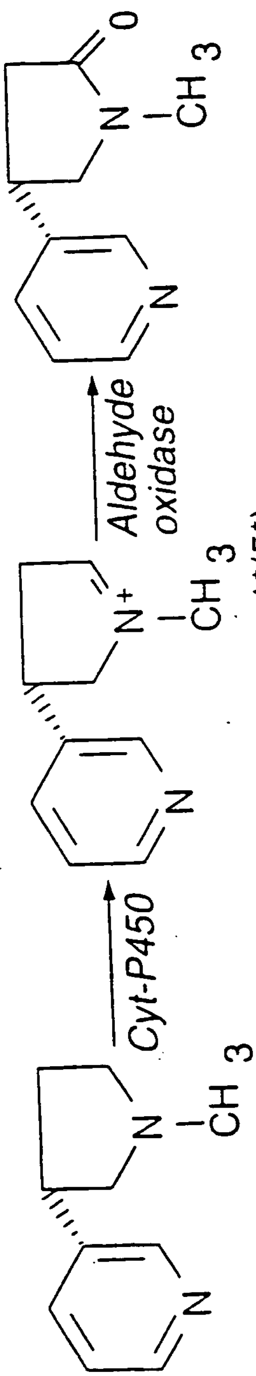


(S)-Nicotine

Nicotine -Δ-1'(5') iminiunion

(S)-Cotinine

FIG.1

FIG.2A

```
LOCUS       HSU22027      7215 bp     DNA          PRI       22-OCT-1995
DEFINITION  Human cytochrome P450   (CYP2A6V2)  gene, complete cds.
ACCESSION   U22027
NID         g1008461
KEYWORDS    .
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 7215)
  AUTHORS   Fernandez-Salguero,P., Hoffman,S.M., Cholerton,S.,Mohrenweiser,H.,
            Raunio,H., Rautio,A., Pelkonen,O., Huang, J.D., Evans, W.E.,
            Idle, J.R. et,al.
  TITLE     A genetic polymorphism in coumarin 7-hydrozylation: sequence of the
            human CYP2A genes and identification of variant CYP2A6 alleles
  JOURNAL   Am. J. Hum. Genet. 57 (3), 651-660 (1995)
  MEDLINE   95397851
REFERENCE   2 (bases 1 to 7215)
  AUTHORS   Fernandez-Salguero,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-MAR-1995)  Pedro Fernandez-Salguero,  National
            Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20894, USA
FEATURES             Location/Qualifiers
     source          1..7215
                     /organism="Homo sapiens"
```

FIG.2A CONT.

```
5'UTR        782..790
CDS          join (791..970, 1237..1399, 2115..2264, 2499..2659,
             3207..3383, 4257..4398,4873..5060,5577..5718, 6308..6489)
             /gene=CYP2A6V2:
             /codon_start=1
             /product=cytochrome P450"
             /db_xref-PID:g1008462"
             /translation=MLASGMLLVALLACLTVMVLMSVWQQRKSKGKLPPGPTPLPFIG
             NYLQLNTEQMYNSLMKISERYGPVFTIHLGPRRVVVLCGHDAVREALVDOAEEFSGRG
             EQATFDWVFKGYGVVFSNGERAKQLLRFAIATLRDFGVGKRGIEERIQEESGFLIEAI
             RSTHGANIDPTFFLSRTVSNVISSIVFGDRFDYKDKEFLSLLRMMLGIFQFTSTSTGQ
             LYEMFSSVMKHLPGPQQQAFQLLQGLEDFIAKKVEHNQRTLDPNSPRDFIDSFLIRMQ
             EEEKNPNTEFYLKNLMMSTLNLFIAGTETVSTTLGYGFLLLMKHPEVEAKVHEEIDRV
             IGKNRQPKFEDRAKMPYMEAVIHEIQRFGDVIPMSLARRVKKDTKFRDFFLPKGIEVF
             PMLGSVLRDLRFFSNPRDFNPQHFLGEKGQFKKRDAFVPFSIRKRNCFGEGLARMELF
             LFFTTVMQNFRLKSSQSPKDIDVSPKHVGFATIPRNYTMSFLPR
exon         791..970
             /gene=CYP2A6V2:
             /number=1
exon         1237..1399
             /gene=CYP2A6V2:
             /number=2
exon         2115..2264
```

FIG.2A CONT.

```
                     /gene=CYP2A6V2:
                     /number=3
     exon            2499..2659
                     /gene=CYP2A6V2:
                     /number=4
     exon            3207..3383
                     /gene=CYP2A6V2:
                     /number=5
     exon            4256..4398
                     /gene=CYP2A6V2:
                     /number=6
     exon            4873..5060
                     /gene=CYP2A6V2:
                     /number=7
     exon            5577..5718
                     /gene=CYP2A6V2:
                     /number=8
     exon            6308..6489
                     /gene=CYP2A6V2:
                     /number=9
     3'UTR           6490..6744
BASE COUNT     1646 a   2196 c   1746 g   1627 t
ORIGIN
```

09/214851

09/214851

FIG.2A CONT.

```
BASE COUNT
   1 aagttcccct gaaatatggc tctggtcttc ctccccttgc caatgaagaa gatggcagtg
  61 gaggttctat ggcagccatc ctggcctcac tctgaggttc caatgaggat tctgggcatc
 121 aagagacagc tctgggcaaa gctaaatcaa gtcagcccct ggacccagtg ctgggctgct
 181 gggctttctg ggagaacgcc gctgggcttg ctacacactc ctcctcccag aaactccaca
 241 cccacagccc tgggtcttcc tagccccgag actttcaagt ccatatgcct ggaatccccc
 301 ttcctgagac ccttaaccct gcatcctcca caacagaaga cccctaaatg cacagccaca
 361 ctttgtctta ccctaataaa acccagacct ttggattcct ctcccctgga acccccagat
 421 ccgcacaact ttggggtgca ttctcactct cagaccccaa atccaaagcc caagtgctcc
 481 cctatgcaaa tattccaaac tcctcagttc tacagcttat ctgttgcccc ctcctaaatc
 541 cacagccctg cggcacccct cctgaagtac cacagattta gtctggaggc cccctctctg
 601 ttcagctgcc ctggggtccc cttatcctcc cttgctggct gtgtcccaag ctaggcagga
 661 ttcatggtgg ggcatgtagt tgggaggtga aatgaggtaa ttatgtaatc agccaaagtc
 721 catccctctt tttcaggcag tataaaggca aaccacccca gccgtcacca tctatcatcc
 781 ctctaccacc atgctggcct cagggatgct tctggtggcc ttgctggcct gcctgactgt
 841 gatggtcttg atgtctgttt ggcagcagag gaagagcaag gggaagctgc ctccgggacc
 901 caccccattg cccttcattg gaaactacct gcagctgaac acagagcaga tgtacaactc
 961 cctcatgaag gtgtcccaag acagggagat gggtgtctcg gggtgggggc tgcctagttg
1021 gctggggctt tgtggcaggg ggttgaccag tgtggaccag agtcttagga aatggagttt
1081 tggagtttca gcatcagaaa gacaggatct tgggatgtcc agctccctga ctgtgagaac
1141 ctgggtgcga agcatcccag cacatgacat ctcggtgctg ggccccattc agagtggagg
1201 gttctccctc taaccactcc cacccacctc catcagatca gtgagcgcta tggccccgtg
1261 ttcaccattc acttggggcc ccggcgggtc gtggtgctgt gtggacatga tgccgtcagg
```

WO 98/03171

PCT/CA97/00506

FIG.2A CONT.

```
1321 gaggctctgg tggaccaggc tgaggagttc agcgggcgag gcgagcaagc caccttcgac
1381 tgggtcttca aaggctatgg tgcccaagag ggggaaggtg ggcaggtgga cacgaaggtc
1441 tcagtgttcc cagccttctc cctgactctc ctgacaactg gaggataagg gagagtcccc
1501 agtctggtct tccctcccca tctccctaca ttggggcctc tccatgtgta tccctcacct
1561 gtctccagcg gccctgtcct gattcctccc tgcctctctc tgccccacct ccttattctc
1621 tctcactgga gtctcctctt tcccctctct tcccctctct ctccatctct tgggtttctg
1681 tttaccagcc ctgggtctct gtctacatga gtctttgagg ccctcttagc ttctgggctt
1741 ctctgggttt ctcatctctc cggatccctt tctcaattct tcctctgtct taggatgcca
1801 gggttattcc tacttccaca tcttcaggct ccatctcctg gtaacagtct ctcttccttc
1861 cagaccctct ctgtttctat ctcaatatta aactctctgc tccagctcag cttaagaatc
1921 tcacaccaag agaggatgtc ctccacccag atctccccat atctcactac cccaccctcc
1981 atcctctgcc tccatcactc tctttctctc cccactgccc tgcggacgcg atccaatgga
2041 gtgtggagct aatgccgtga agctatgtgc atctctctgt ctggccgtac ctgggtaata
2101 acctgatcga ctaggcgtgg tattcagcaa cggggagcgc gccaagcagc tcctgcgctt
2161 tgccatcgcc accctgaggg acttcggggt gggcaagcga ggcatcgagg agcgcatcca
2221 ggaggagtcg ggcttcctca tcgaggccat ccggagcacg cacggtgagc aggggacccc
2281 gagtgcgggg gcaggagaag gaaaacaccc aggacgagga acccgcgcgc gttctgcctg
2341 gggatgggga ctaggtgggg aaaggcgccc gcacttccag ccctggagtc tggcgctggg
2401 aatttggctc aacaaggccc tgcctcctgg aattctgact ctcctcagac ctctgagttg
2461 actctctccc caaccccctt ctcccgacat acccggaggc gccaatatcg atcccacctt
2521 cttcctgagc cgcacagtct ccaatgtcat cagctccatt gtctttgggg accgctttga
2581 ctataaggac aaagagttcc tgtcactgtt gcgcatgatg ctaggaatct tccagttcac
2641 gtcaacctcc acggggcagg taatggttgc agcccggccc gtgaaggccc ttaccaaaac
```

09/214851

FIG.2A CONT.

```
2701 cggcaaattg ttcccctacc gggggaaggg ggccccaaat tcccaccgcc ccccggacag
2761 tgtcccctca aaatcagtcc ccgatttggg caaattggca gagtggaacc agacccgggt
2821 tggttgtcca atcccctgct ctccagggac accgggatag cacaacagat gctccccaaa
2881 acagagcctg ctggcaggat gcataccctc agctcagctc tctcaccctg ggcacgtgtt
2941 cccatcccca acttaccggt aatttctaac agatgctccc tacccaggtc ttcttgaata
3001 ttttaacacc cggaaaccct gggtacctaa ccttcctgt  aaactttaga gattagttcc
3061 tatccggccc ctctgaaata cctaaccacc ggagaccaga tgcctttaac tcagttcctt
3121 ccttgctatg aaacaaatcc cattcccatc agctcctgcc ccgtgacagc tgtccttccc
3181 ttcccatcct ctctctgcaa ccccagctct atgagatgtt ctcttcggtg atgaaacacc
3241 tgccaggacc gcagcaacag gcctttcagt tgctgcaagg gctggaggac ttcatagcca
3301 agaaggtgga gcacaaccag cgcacgctgg atcccaattc cccacgggac ttcattgact
3361 cctttctcat ccgcatgcag gaggtacacc ccagcagcca ctgcggggag atgcaaagcc
3241 aggcagaggg aaatcagtct gggagtgggg caggcagatg acacaggccc attcaaatta
3481 accctcatca taataatcct cacaattggc tgggtgccgt ggctaacagc ctgtaatccc
3541 agcactttgg gaggccgagg caggtggatc acctgaggtc aggagttcga gaccagcctg
3601 gccaacatgg tcaaaccccg tctctactaa aaatccaaaa attagttggg catggtggcg
3661 cgaagggggg cagaggttgc aatgagccaa gatcacggca ttgcactcca gtctgggtga
3721 cagaatgagg ccctgtgtca aaaaaaatta atcacttgtt taaaaagtaa gtgagcctgc
3781 atggtcatgc gcatgtgcag ctccagctac tcaggaggct gaggctggag gattgcttga
3841 gctcaggagt tggcgtccgg cctgtgcaac ttagcaagac caagtcagta taagaaaaaa
3901 aaaaaacaaa aaaaaagctg acagctaagt tgataattga cggacagatg gtcagcaagg
3961 taacgaaggt gagaaggaag agcattgggg gcaacgccag gagtcagggc aagggctggt
```

09/214851

FIG.2A CONT.

```
4021 tcctagagcg agtctggtag gatctagggc ccctcttctc caccctgcgg tcttgcccca
4081 aagagaggtc gagggtgctg ggattgcgct agactcgagt ctgtgtagat cttggggtcc
4141 cctcttgacc cccattggtc tgaacctaag agtggaagat ccatggggtg aaccccctaga
4201 tggtgccctg aggtcaagca ggagtgaggt tgtcctaaag ccccctctcc cttcaggagg
4261 agaagaaccc caacacggag ttctacttga agaacctgat gatgagcacg ttgaacctct
4321 tcattgcagg caccgagacg gtcagcacca ccctgcacta tggcttctta ctgctcatga
4381 agcacccaga ggtggagggt aaggctggag ggggacggaa gtggagggcc ccagaccctc
4441 aaaattcccc ttcgactggt gcaatgtccc cacctgtccc agatcccggg accctgagac
4501 gtgacttgct gtccagagac agggcaacat tcagctggta ggcatcagct gagtctcatt
4561 agatattaaa atattgaaaa tgtctgcact gattggtcag tcacttctgt cccaagccca
4621 ctgagtgccc actgcccgtt ccaccgggtc atcccctaag ttcctccctg tgcctcccct
4681 gtgattctgg cacaacctgg ttaacaggat cctactccaa caatgcgaat gggtgatgtc
4741 tgttctgtta tgaatgctct acttccgtct cataggcgga ggcatttcat ccaccccatt
4801 ttgcctatcc ggactatcat ttcctgctct gagaccccta gatacctaaa cacattcccc
4861 ctcctccccc agccaaggtc catgaggaga ttgacagagt gatcggcaag aaccggcagc
4921 ccaagtttga ggaccgggcc aagatgccct acatggaggc agtgatccac gagatccaaa
4981 gatttggaga cgtgatcccc atgagtttgg cccgcagagt caaaaaggac accaagtttc
5041 gggatttctt cctccctaag gtgctatccg cccccacccc ccagactacg gggactccag
5101 cccctctctg tgtccccagc atcccaccca cattagaagc tttctagacc ctgtcccact
5161 ccctcaatca gtcaaaaaag acttccccaa ccaccacatc cgttccacct ttccacttag
5221 acactcctga gtcctgcatc tctccagact ctttgtgtca ggagaatcaa acacatgttc
5281 ccaaacttcc tatcttaaga aacagaagcc cccttttccat tcggcctttt gtcatagggga
5341 cagaaatctc aggtccccca aactcctgcc tagaaggaca tggaccccat gtctcccaaa
```

FIG.2A CONT.

```
5401 cttcctgttt cagagatgtg aaccttctat cccccaaggt cctccctcag aggtccccaa
5461 ttcccatgcc tgccacttcc cctcaccggg gcaccctagt tcccctcca gcccctgtgt
5521 actctcaaca atcccccaac ccgcctcatc acatacacct tcctcctccc tcccagggca
5581 tagaagtgtt ccctatgttg ggctccgtgc tgagagacct caggttcttc tccaaccccc
5641 gggacttcaa tccccagcac ttcctgggtg agaaggggca gtttaagaag cgtgatgctt
5701 ttgtgccctt ctccatcagt aagagaccac tgtttggtgc caggcttact actcacacca
5761 gcaggggcct cccttaccca gttcccctct ctgccgtgta gcctagtatt tccccagctt
5821 ggcaagttcc tgttagcaat ctaccgtcga gccaccaggt gatactccct taactaccaa
5881 gcacccagta cctgtgccca ggcaaaagga aaggaaacat catacccctt tcagaggcgg
5941 gggaaaacca aaggccagag agaatcagag atttatttcc ctagggtcac acaggagatt
6001 cttcagcatc cctaaaaagg agatgacggc acagcaggtc atatttggga gttcttatct
6061 gggggaaggg ggatcttaaa cctcccattg tggacacctg gcatcgatca accccatctt
6121 ttggtcatct tttgggtcac tcaaggaaac tgaggtcaag gagggtcaag aggctccctc
6181 ttaaagtctc tcagggccat atattccacc cttcctccct gggagagccg cagctggagg
6241 tcggtactgg ggcgaggctg cactgagagt gggcttcacc tccacccctc ccgcctctcc
6301 tcctcaggaa agcggaactg tttcggagaa ggcctggcca gaatggagct ctttctcttc
6361 ttcaccaccg tcatgcagaa cttccgcctc aagtcctccc agtcacctaa ggacattgac
6421 gtgtccccca aacacgtggg ctttgccacg atcccacgaa actacaccat gagcttcctg
6481 ccccgctgag cgagggctgt gccggtgaag gtctggtggg cggggccagg gaaagggcag
6541 ggccaagacc gggcttggga gaggggcgca gctaagactg gggcaggat ggcggaaagg
6601 aaggggcgtg gtggctagag ggaagagaag aaacagaagc ggctcagttc accttgataa
6661 ggtgcttccg agctgggatg agaggaagga aacccttaca ttatgctatg aagagtagta
```

FIG.2A CONT.

```
6721 ataatagcag ctcttatttc ctgagcacgt acccccgtgt cacctttgtt caaaaaccat
6781 tgcacgctca cctaatttgc cacaaaaccc ccttcgaagg ggcgttcatg cccattttac
6841 acgtgacaaa actgaggctt agaaagttgt ctctgatgtc tcacaaaaca taagtgccca
6901 gaaaatctgc gaacacagat ctgtgcccat agccttctag acagattctt aaaaagcacc
6961 tattcctcac gcaaaacagt ttagtataga atcacatggc ctgaacatcc ctgtccgggg
7021 gagttcccca gagacctggg gggtggttgc cctgccttca ctgcacacat gcccacactc
7081 tcacctactc aacatgctgt gactacccgg gtgtaatctg tgcttgctac cagataaggc
7141 cactgtagcc cattcagagt cagcccaggg acacaacgag acatgactgg acatacaggg
7201 tcagtccatt aacaa
```

FIG.2B

```
LOCUS       HSP452B6     1415  bp   RNA           PRI            29-MAY-1992
DEFINITION  Human MRNA FOR CYTOCHROME P-450IIVB6.
ACCESSION   X13494
NID         g35206
KEYWORDS    Cytochrome; cytochrome P450IIB6.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 1415)
  AUTHORS   Miles, J.S.
TITLE       Direct Submission
  JOURNAL   Submitted (10-NOV-1988) Miles J.S., Imperial Cancer Research Fund,
            Lab of Molecular Phrmacology and Drug Metabolism, Hugh Robson
            Building, George Square, Edinburgh, EH8 9XD
REFERENCE   2 (bases 1 to 1415)
  AUTHORS   Miles, J.S., McLaren, A.Q. and Wolf, C.R.
  TITLE     Alternative splicing in the human cytochrome P450IIB6 gene
            generates a high level of aberrant messages
  JOURNAL   Nucleic Acids Res. 17 (20), 8241-8255 (1989)
  MEDLINE   90045947
COMMENT     The sequence is a compilation of genomic and cDNA clones. **map:
            chromosomal location=19q12-13.2;
            Data kindly reviewed (13-NOV-1989) by Miles, J.S.
FEATURES             Location/Qualifiers
```

09/214851

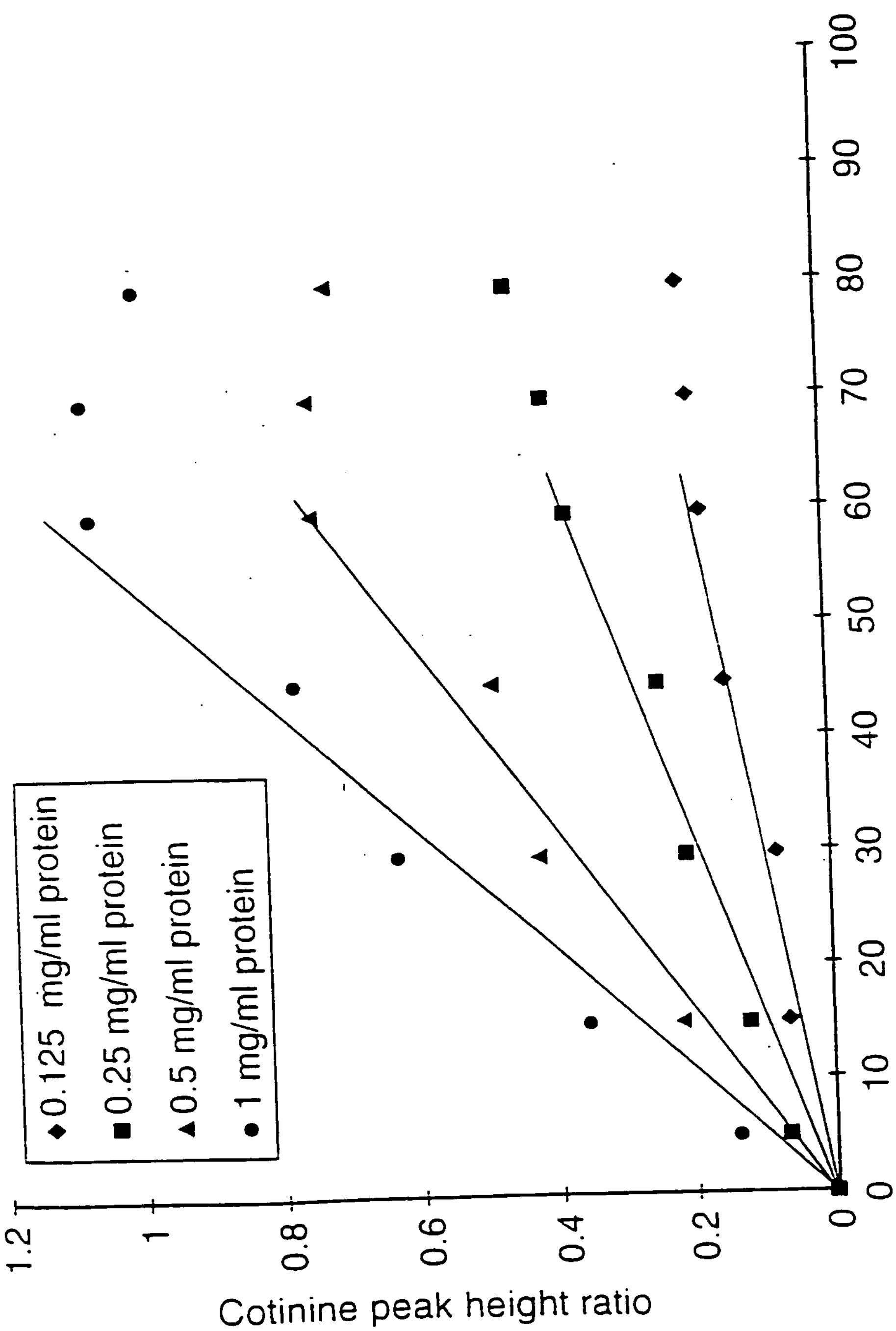
FIG.2B CONT.

```
source          1..1415
                /organism="Homo sapiens"
misc_feature    9..110
                /note=exon 1, partial"
misc_feature    111..273
                /note=exon 2"
misc_feature    274..423
                /note=exon 3"
misc_feature    424..584
                /note=exon 4"
misc_feature    585..761
                /note=exon 5"
misc_feature    762..903
                /note=exon 6"
misc_feature    904..1091
                /note=exon 7"
misc_feature    1092..1233
                /note=exon 8"
misc_feature    1234..1415
                /note=exon 9", coding region"
BASE COUNT      341 a    430 c    328 g    316 t
ORIGIN
      1 gaattccgcc ctgcacccat gaccgcctcc caccagggcc ccgccctctg cccctttgg
```

FIG.2B CONT.

```
  61 gaaaccttct gcagatggat agaagaggcc tactcaaatc ctttctgagg ttccgagaga
 121 aatatgggga cgtcttcacg gtacacctgg gacccaggcc cgtggtcatg ctgtgtggag
 181 tagaggccat acgggaggcc cttgtggaca aggctgaggc cttctctggc cggggaaaaa
 241 tcgccatggt cgacccattc ttccggggat atggtgtgat ctttgccaat ggaaaccgct
 301 ggaaggtgct tcggcgattc tctgtgacca ctatgaggga cttcgggatg ggaaagcgga
 361 gtgtggagga gcggattcag gaggaggctc agtgtctgat agaggagctt cggaaatcca
 421 agggggccct catggacccc accttcctct tccagtccat taccgccaac atcatctgct
 481 ccatcgtctt tggaaaacga ttccactacc aagatcaaga gttcctgaag atgctgaact
 541 tgttctacca gactttttca ctcatcagct ctgtattcgg ccagctgttt gagctcttct
 601 ctggcttctt gaaatacttt cctggggcac acaggcaagt ttacaaaaac ctgcaggaaa
 661 tcaatgctta cattggccac agtgtggaga agcaccgtga aaccctggac cccagcgccc
 721 ccaaggacct catcgacacc tacctgctcc acatggaaaa agagaaatcc aacgcacaca
 781 gtgaattcag ccaccagaac ctcaacctca acacgctctc gctcttcttt gctggcactg
 841 agaccaccag caccactctc cgctacggct tcctgctcat gctcaaatacсctcatgttg
 901 cagagagagt ctacagggag attgaacagg tgattggccc acatcgccct ccagagcttc
 961 atgaccgagc caaaatgcca tacacagagg cagtcatcta tgagattcag agattttccg
1021 accttctccc catgggtgtg ccccacattg tcacccaaca caccagcttc cgagggtaca
1081 tcatccccaa ggacacagaa gtatttctca tcctgagcac tgctctccat gacccacact
1141 actttgaaaa accagacgcc ttcaatcctg accactttct ggatgccaat ggggcactga
1201 aaaagactga agcttttatc cccttctcct tagggaagcg gatttgtctt ggtgaaggca
1261 tcgcccgagc ggaattgttc ctcttcttca ccaccatcct ccagaacttc tccatggcca
1321 gccccgtggc cccagaagac atcgatctga caccccagga gtgtggtgtg ggcaaaatac
1381 ccccaacata ccagatccgc ttcctgcccc gctga
```

09/214851



Protein-time curves of cotinine production from 100 μM nicotine in the presence of 20 μl rat cytosol by K20 human liver microsomes.

FIG.3

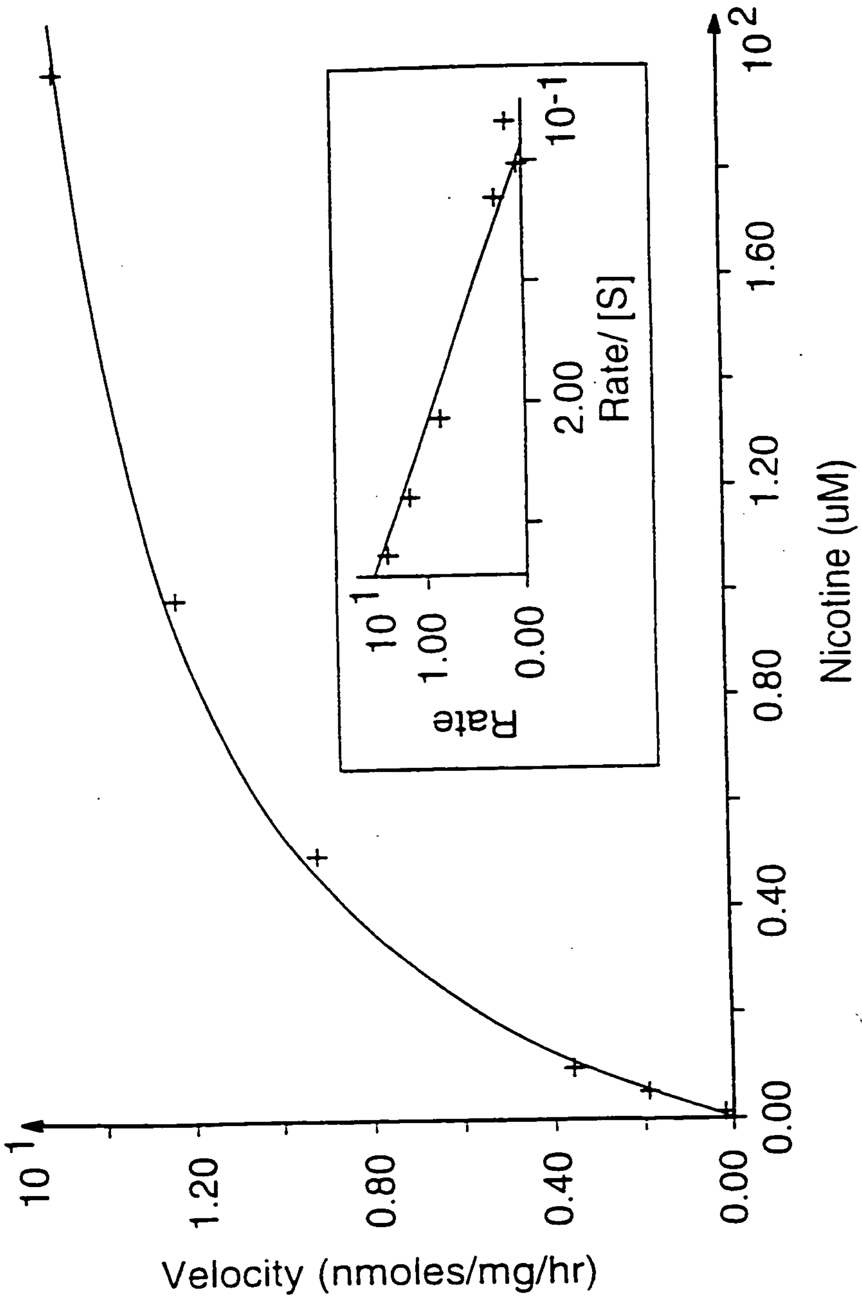

Velocity (nmoles/mg/hr)
10 1
1.20
0.80
0.40
0.00
Nicotine (uM)
0.00
0.40
0.80
1.20
1.60
10 2
Rate
10 1
1.00
0.00
Rate/[S]
2.00
10-1

FIG.4A

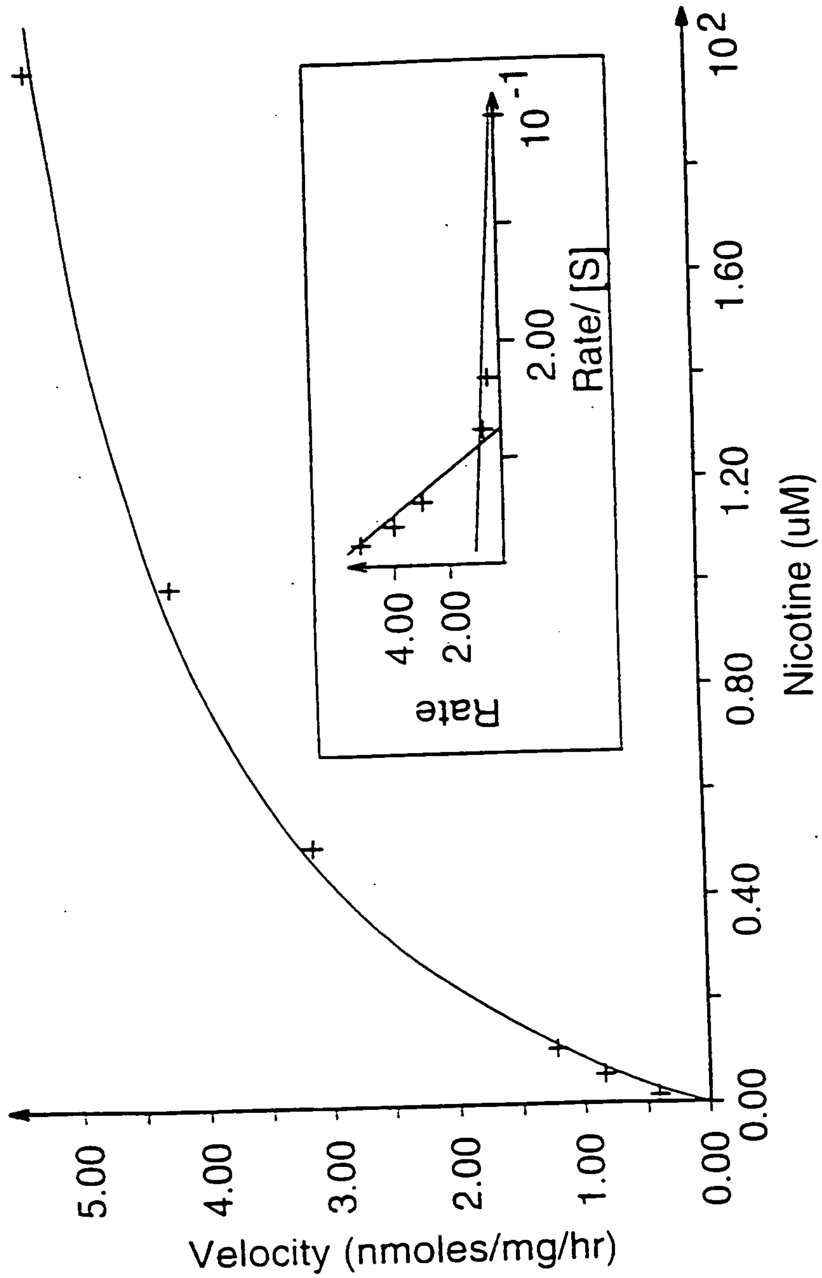

Velocity (nmoles/mg/hr)
5.00
4.00
3.00
2.00
1.00
0.00
0.00
0.40
0.80
1.20
1.60
10^2
Nicotine (uM)
Rate
4.00
2.00
2.00
10^{-1}
Rate/ [S]

FIG.4B

09/214851

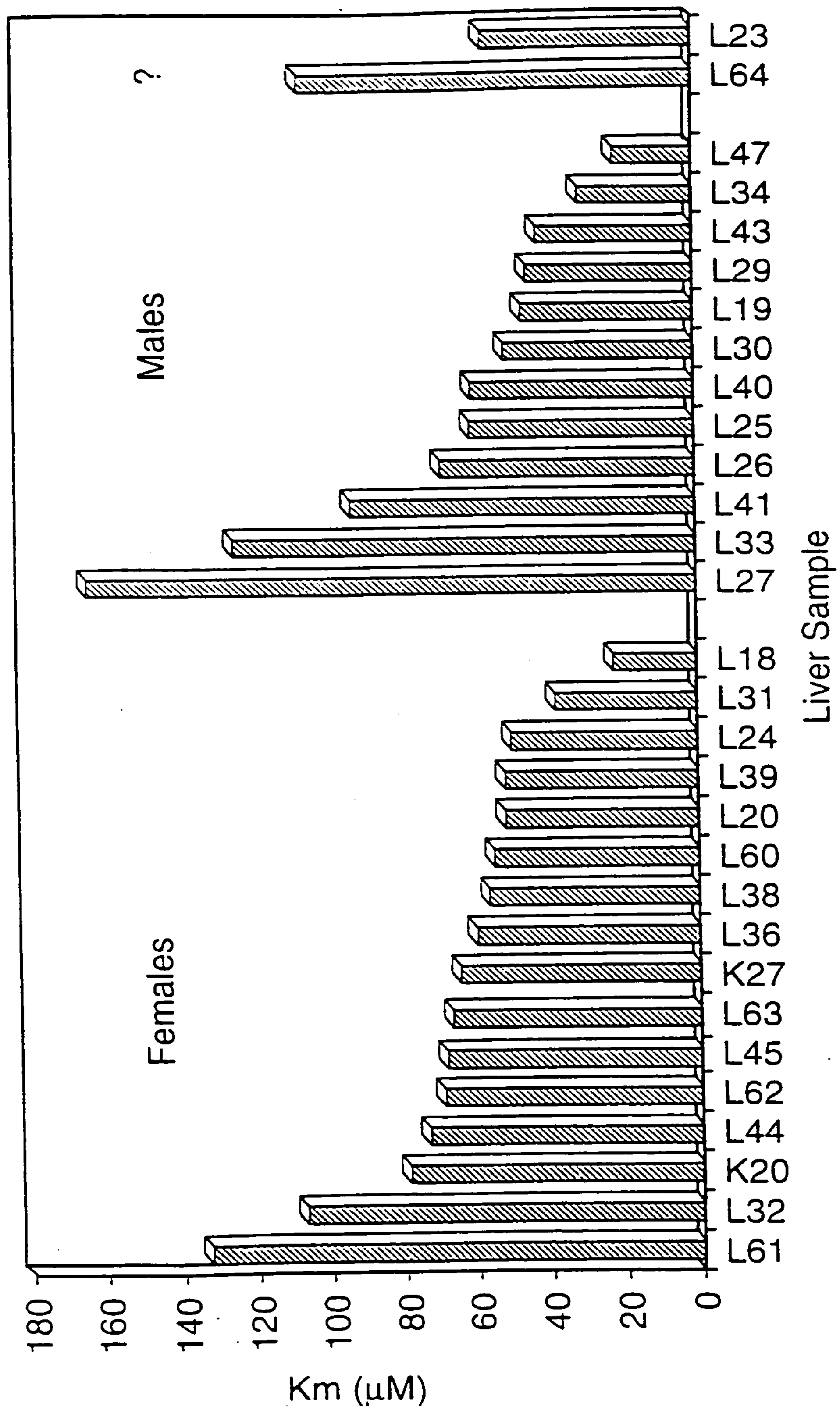


FIG.5

09/214851

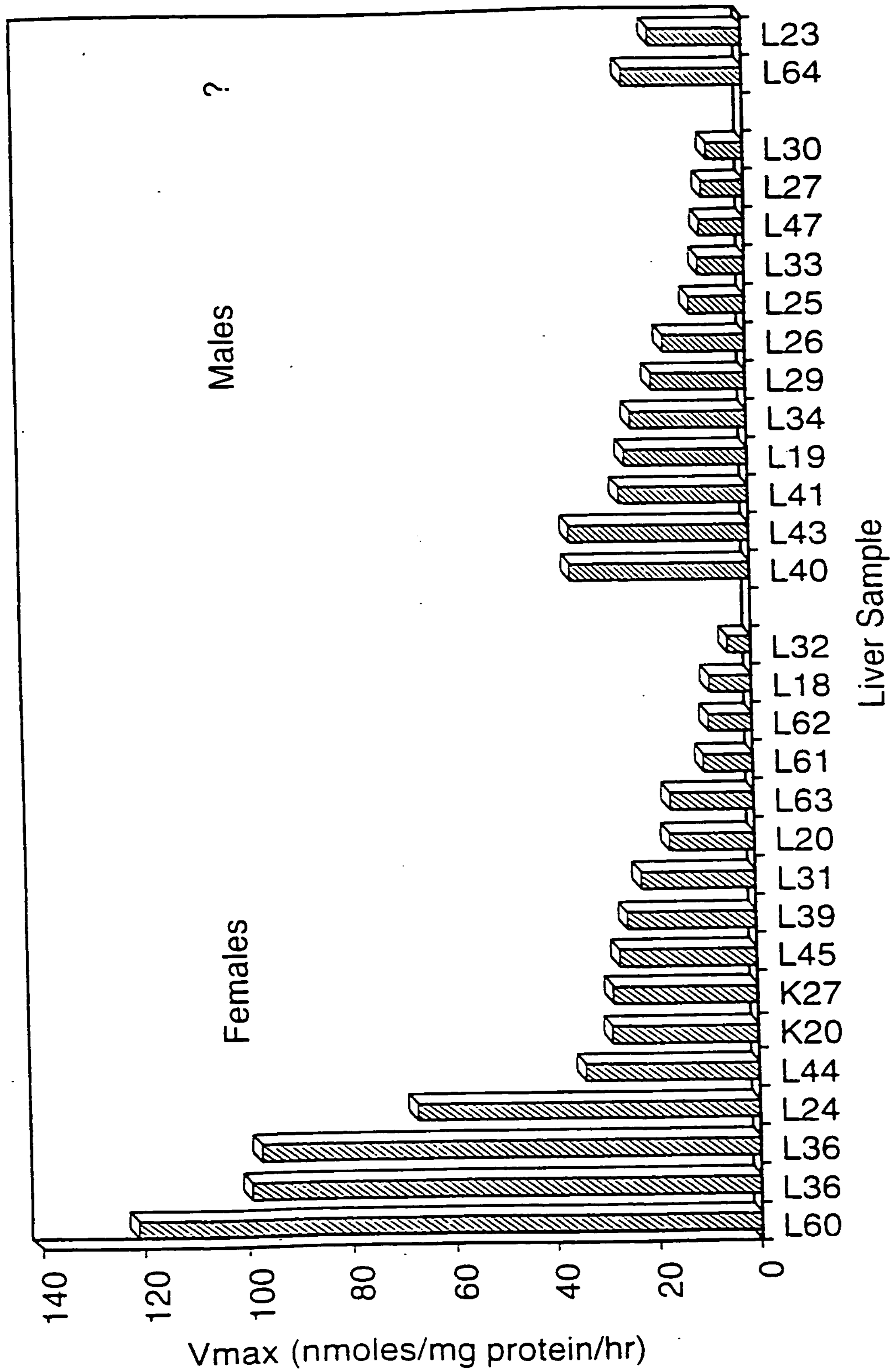


FIG.6

09/214851

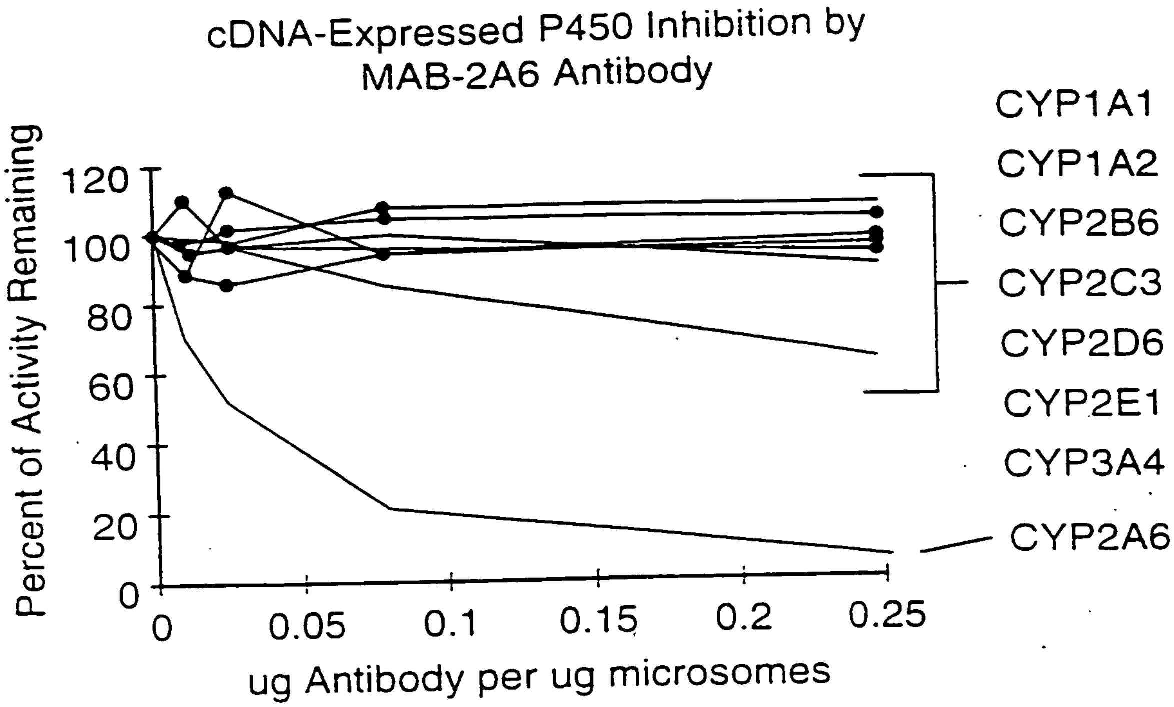


FIG.7A

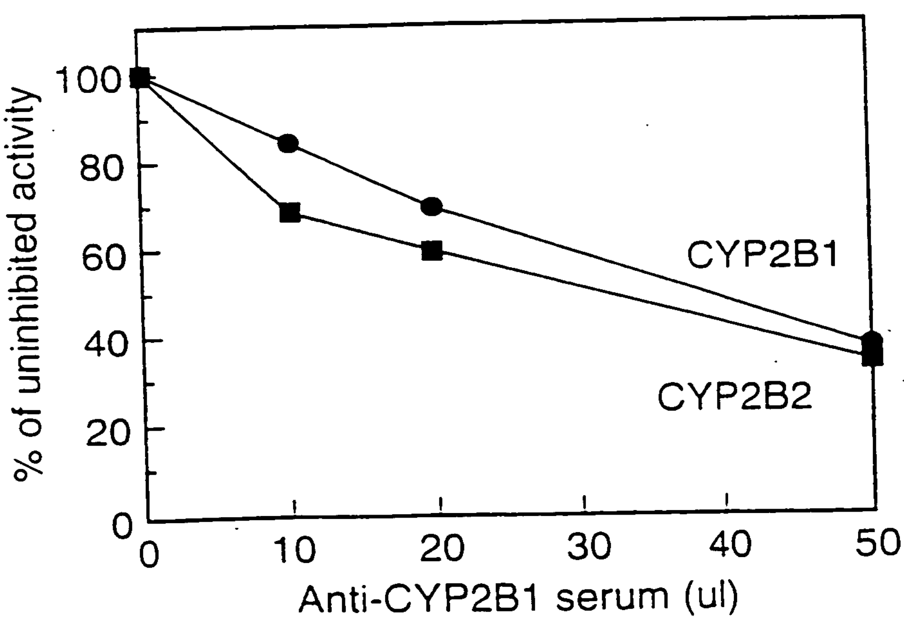


FIG.7B

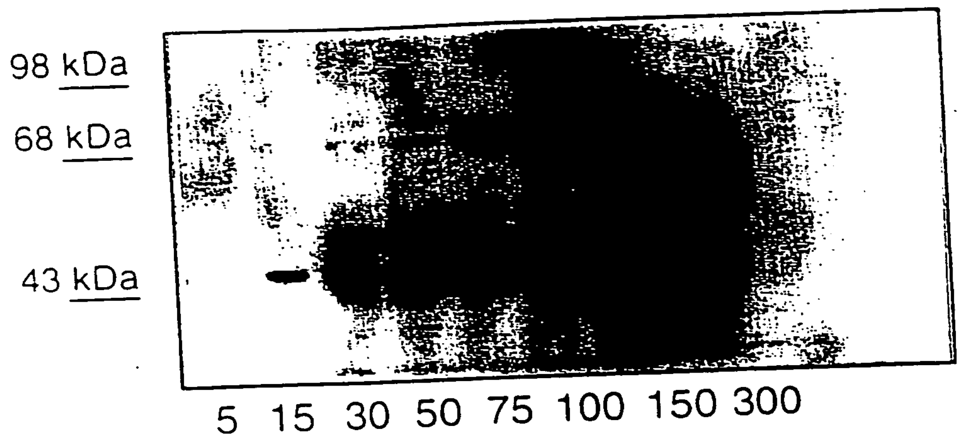

98 kDa
68 kDa
43 kDa
5 15 30 50 75 100 150 300

FIG.8A

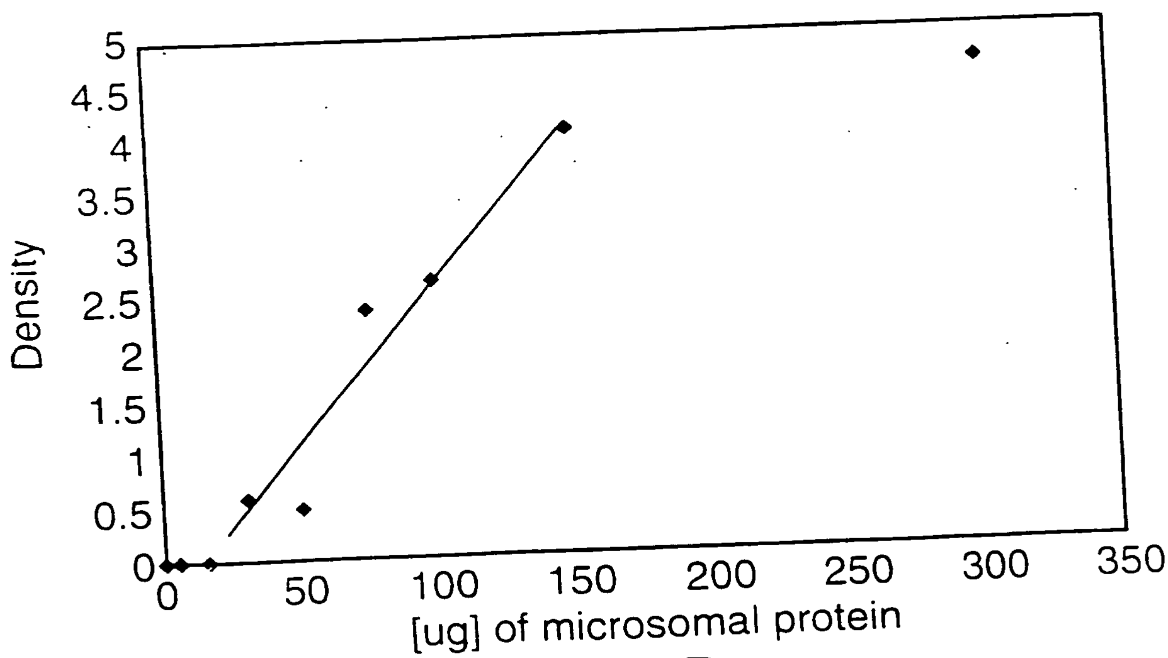

Density
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
300
350
[ug] of microsomal protein

FIG.8B

09/214851

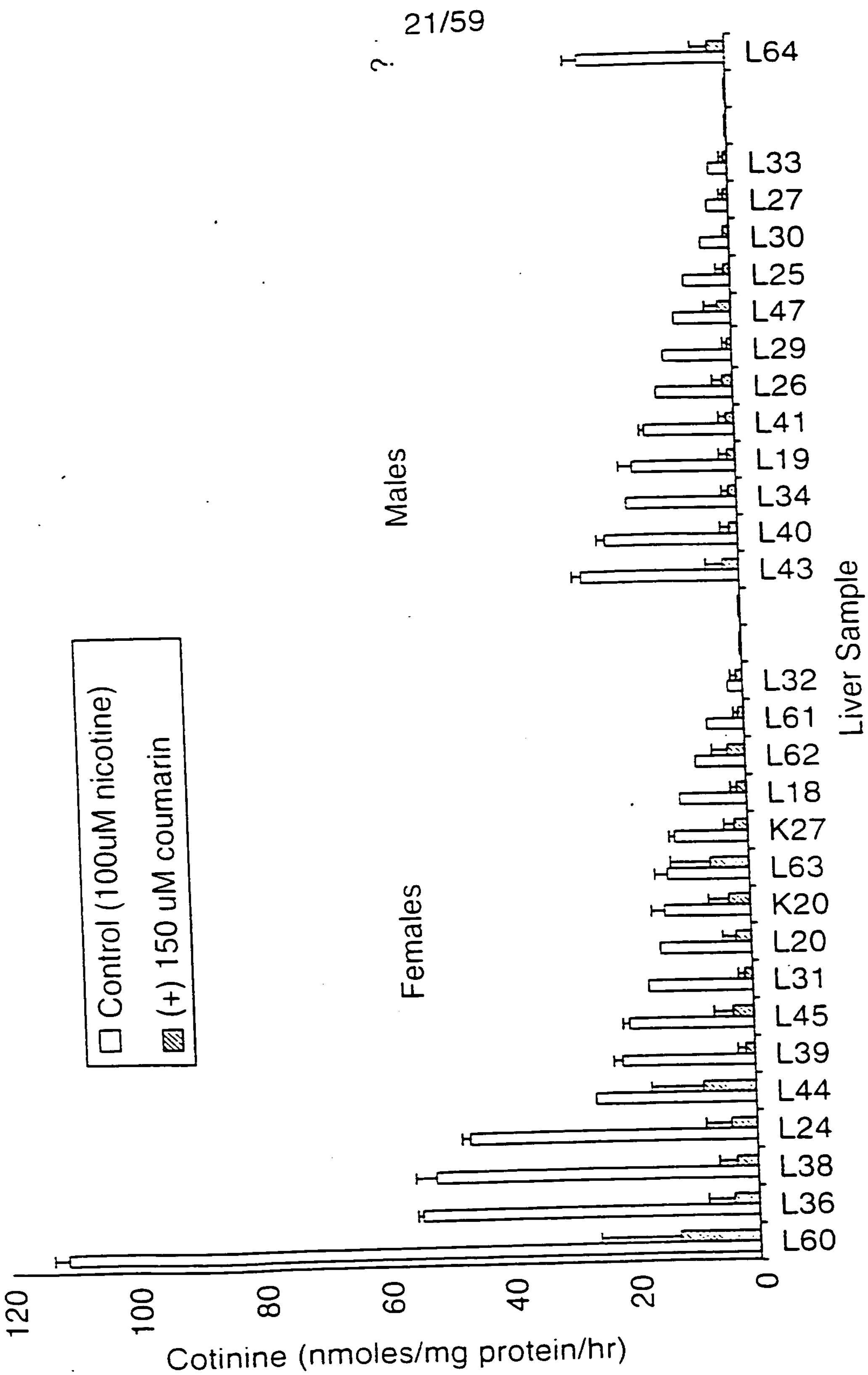


FIG.9

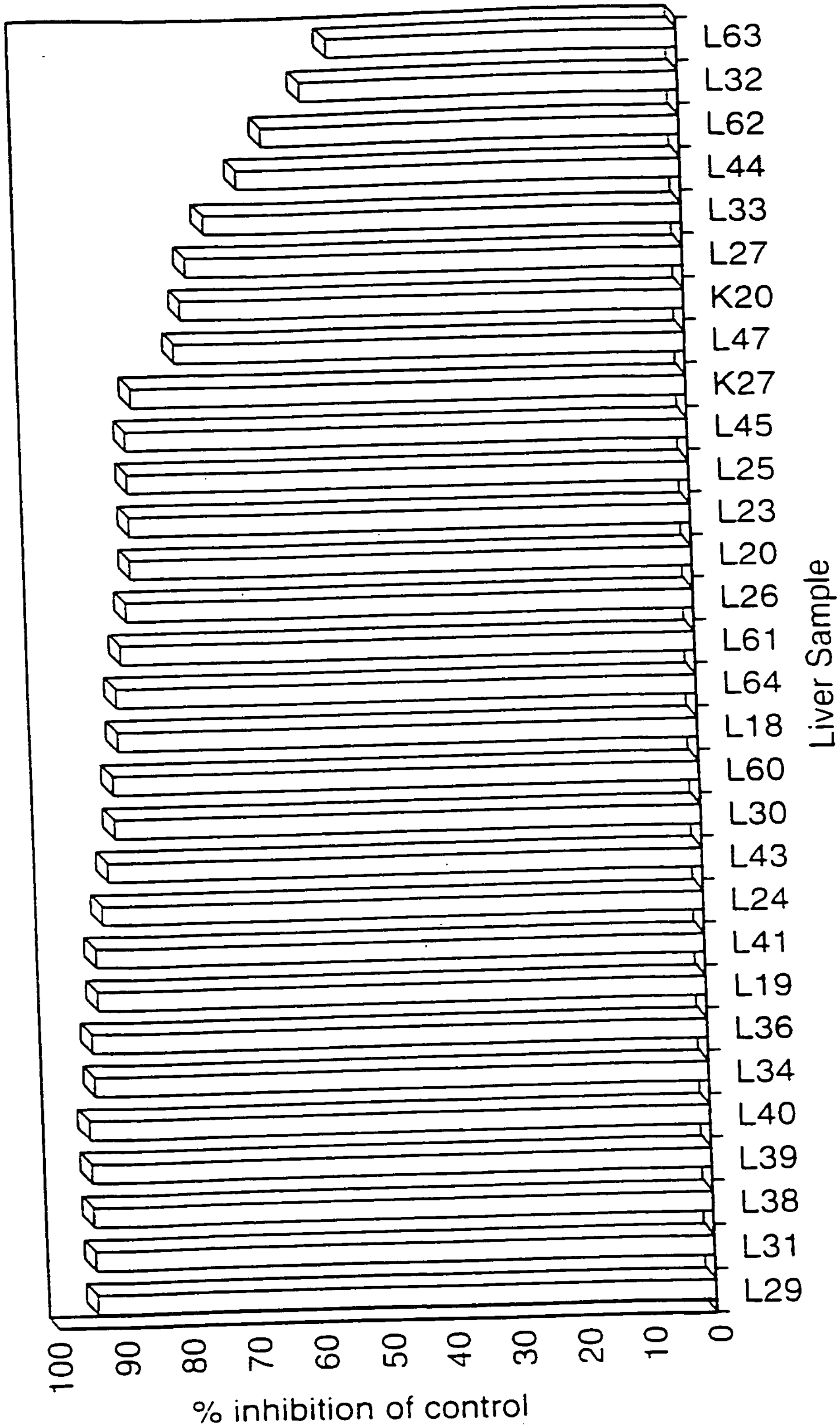
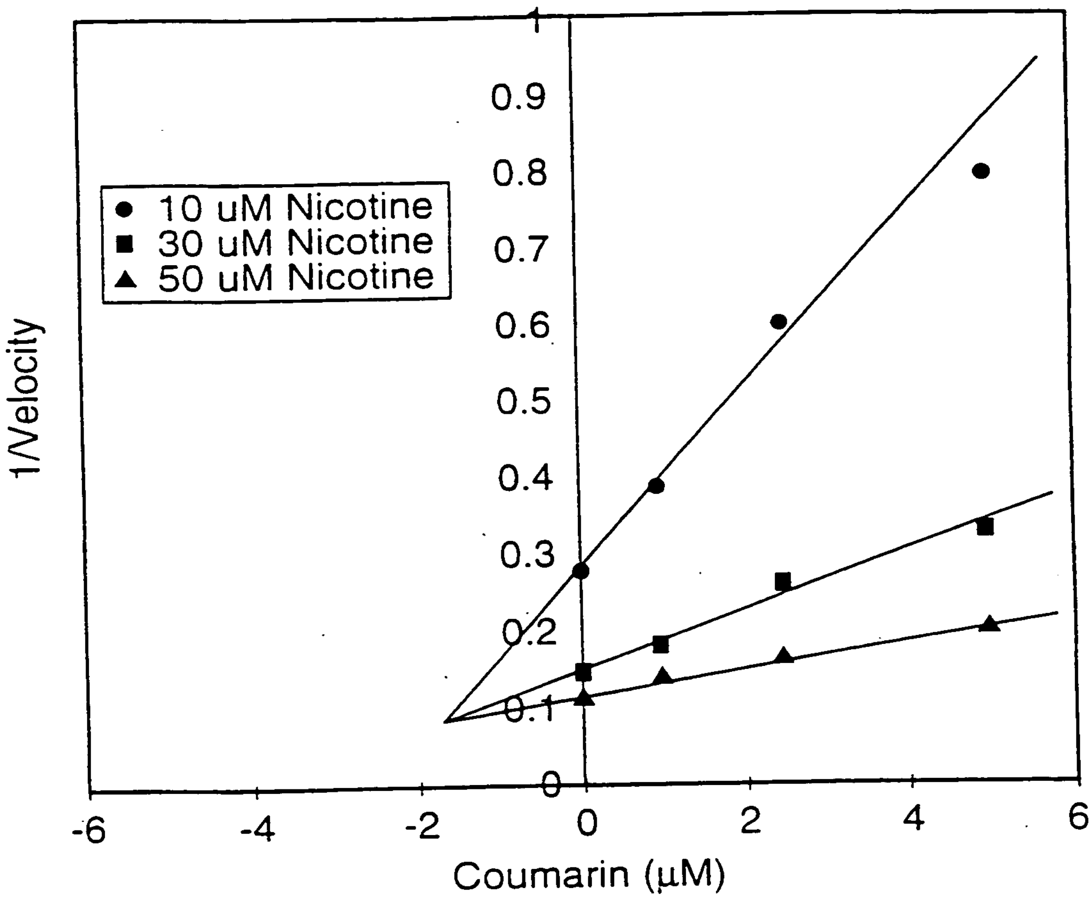

L63
L32
L62
L44
L33
L27
K20
L47
K27
L45
L25
L23
L20
L26
L61
L64
L18
L60
L30
L43
L24
L41
L19
L36
L34
L40
L39
L38
L31
L29
Liver Sample
100
90
80
70
60
50
40
30
20
10
0
% inhibition of control

FIG.10



Ki=1.8 μM

FIG.11

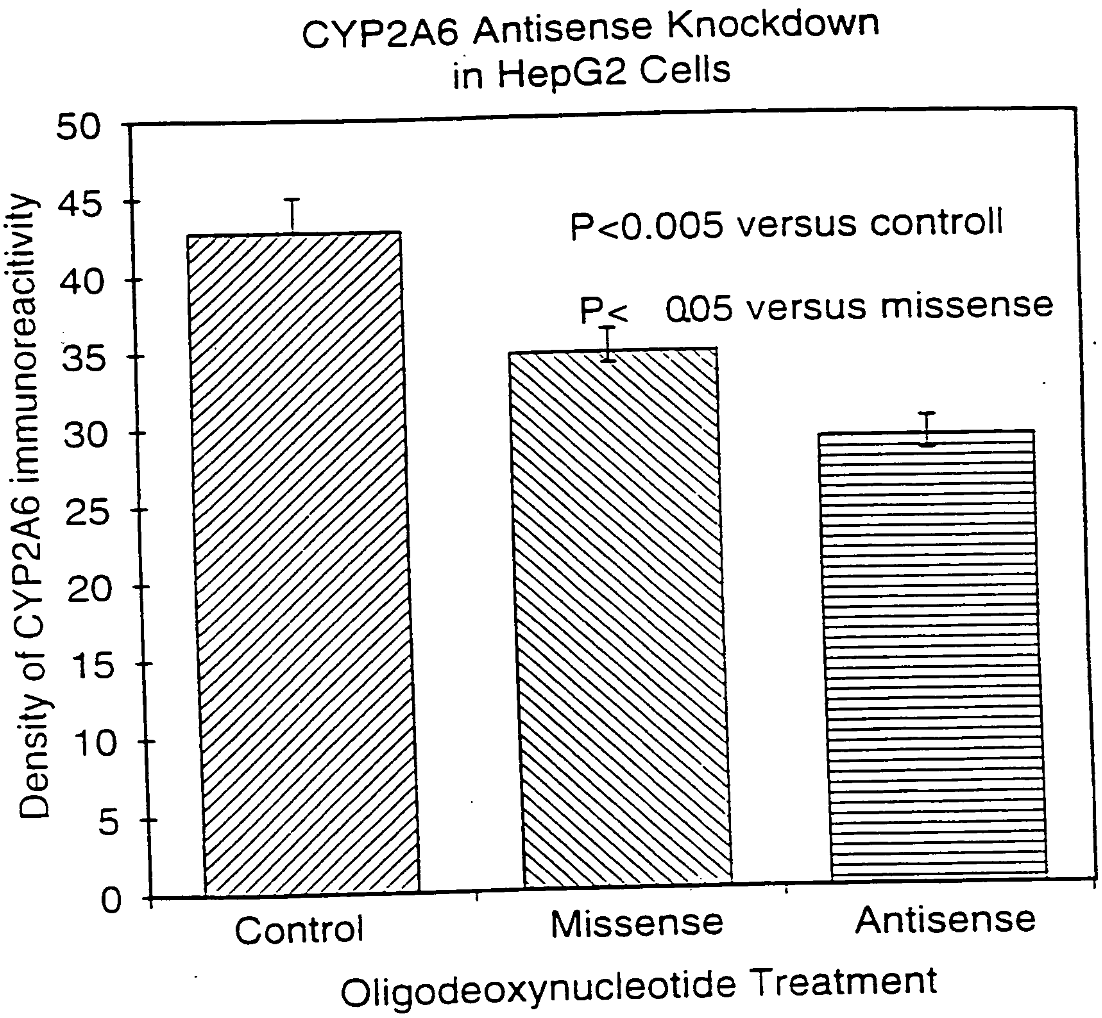

CYP2A6 Antisense Knockdown
in HepG2 Cells
Density of CYP2A6 immunoreacitivity
50
45
40
35
30
25
20
15
10
5
0
P<0.005 versus controll
P< 0.05 versus missense
Control
Missense
Antisense
Oligodeoxynucleotide Treatment

FIG.12

09/214851

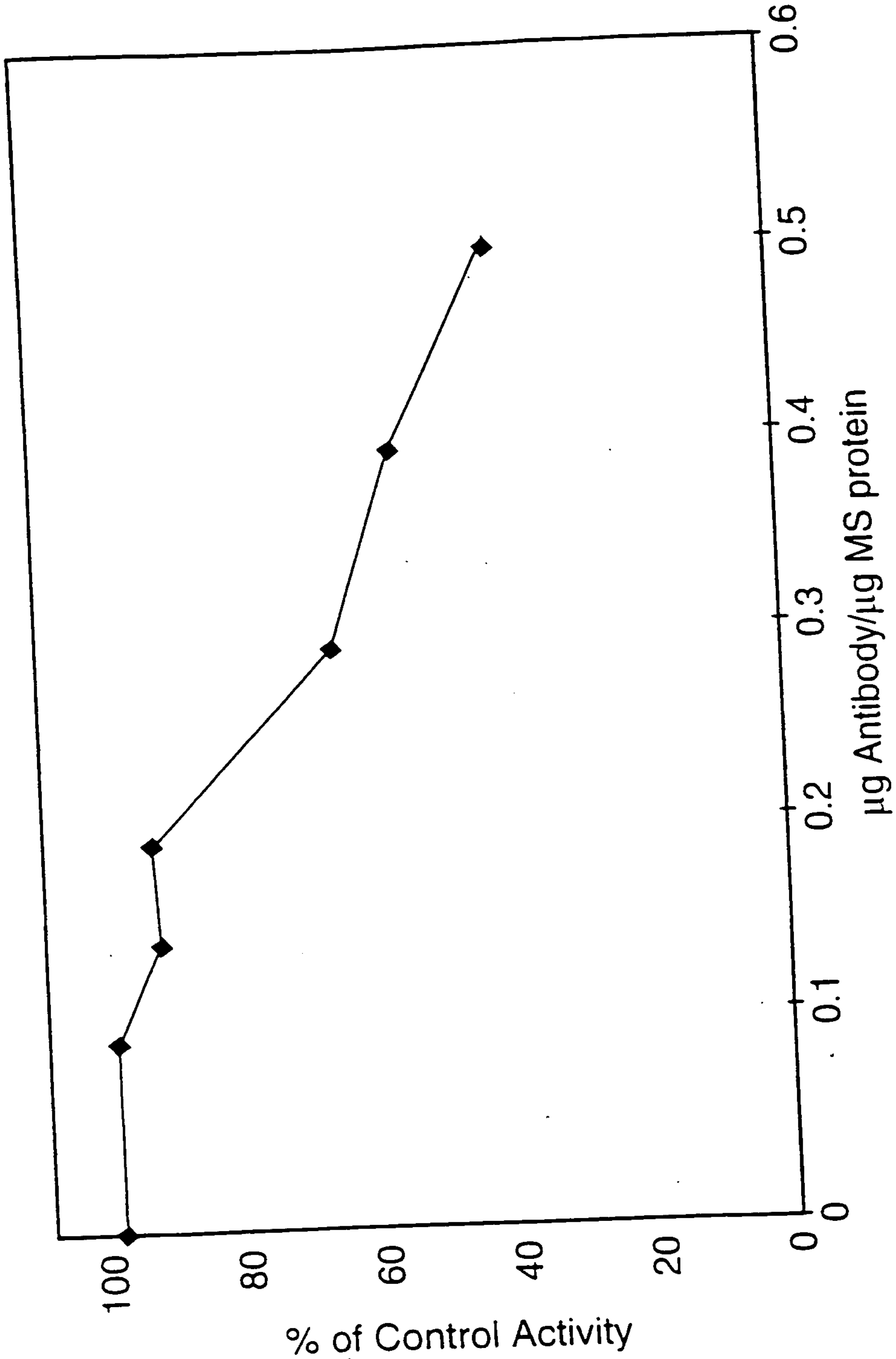


FIG.13

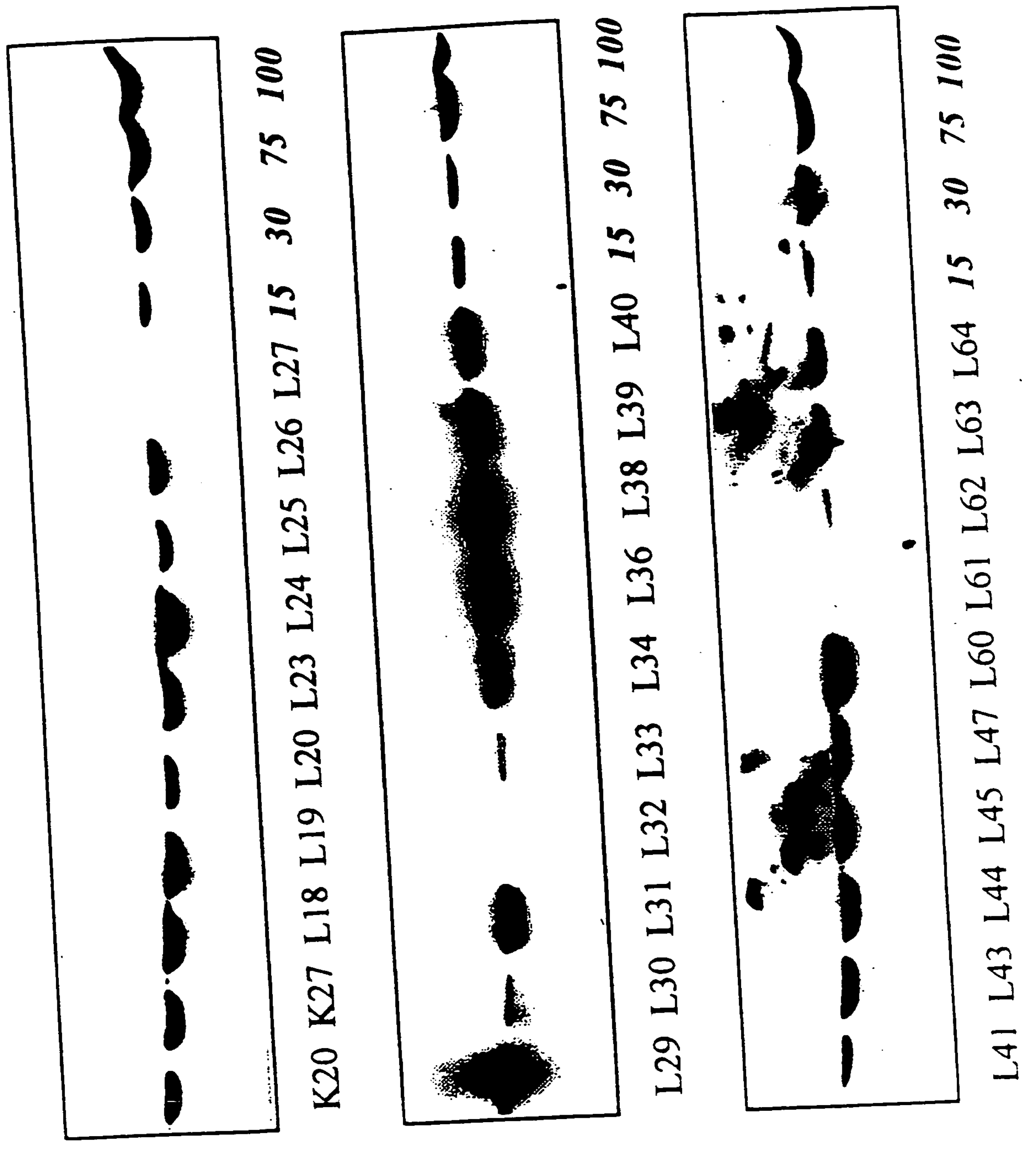


FIG.14A.

FIG.14B.

FIG.14C.

09/214851

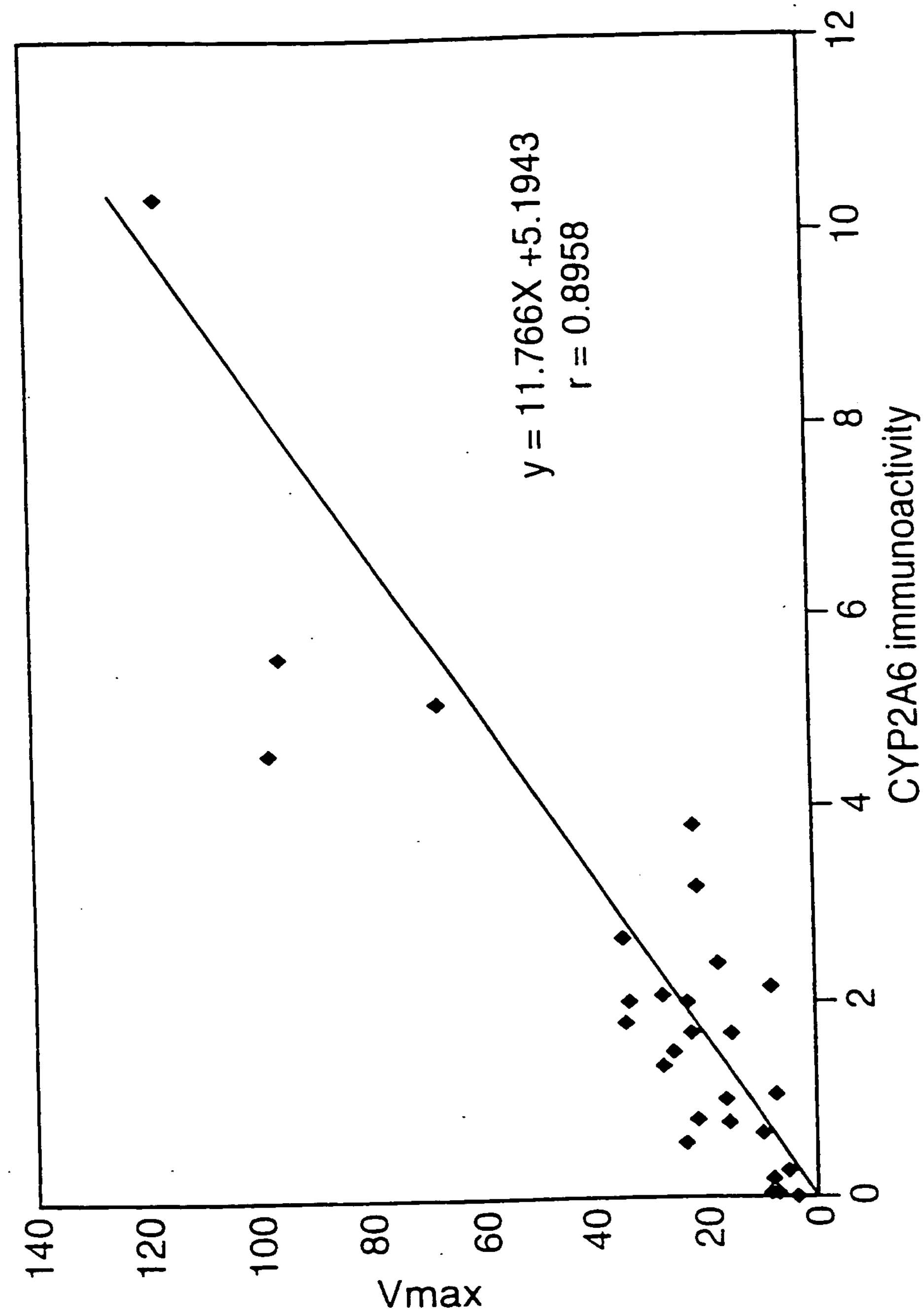


FIG.15

09/214851

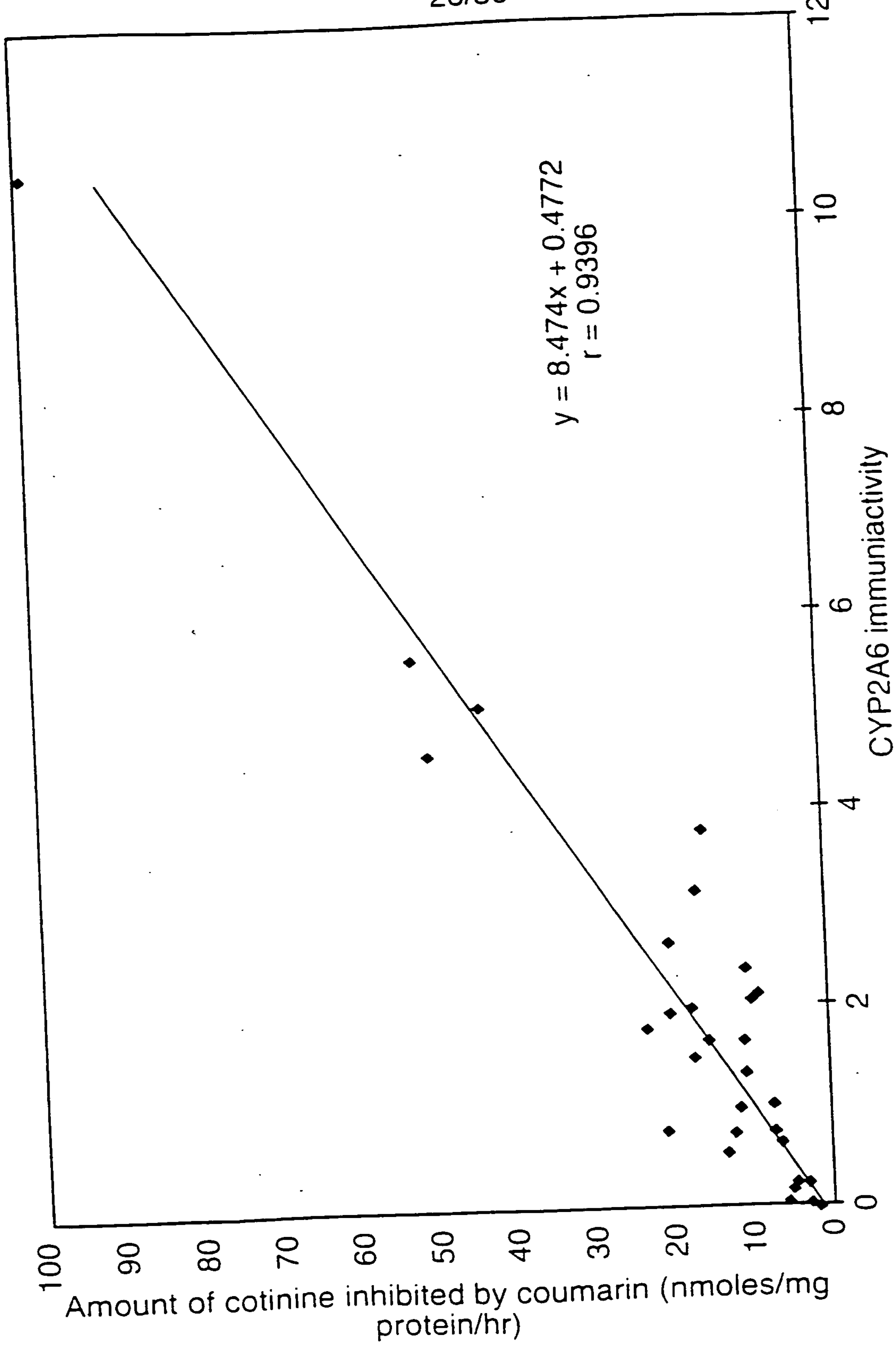


FIG.16

09/214851

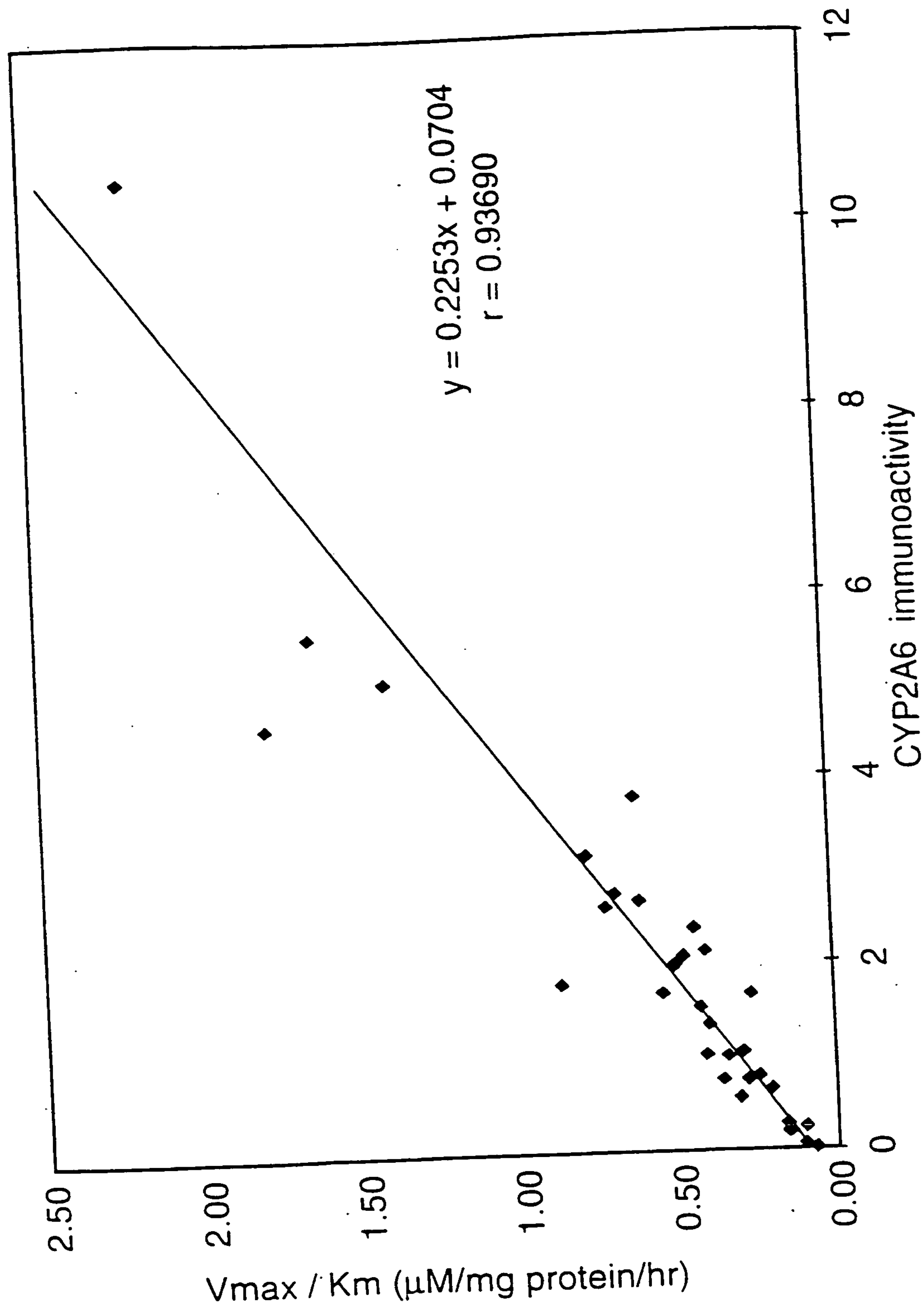


FIG.17

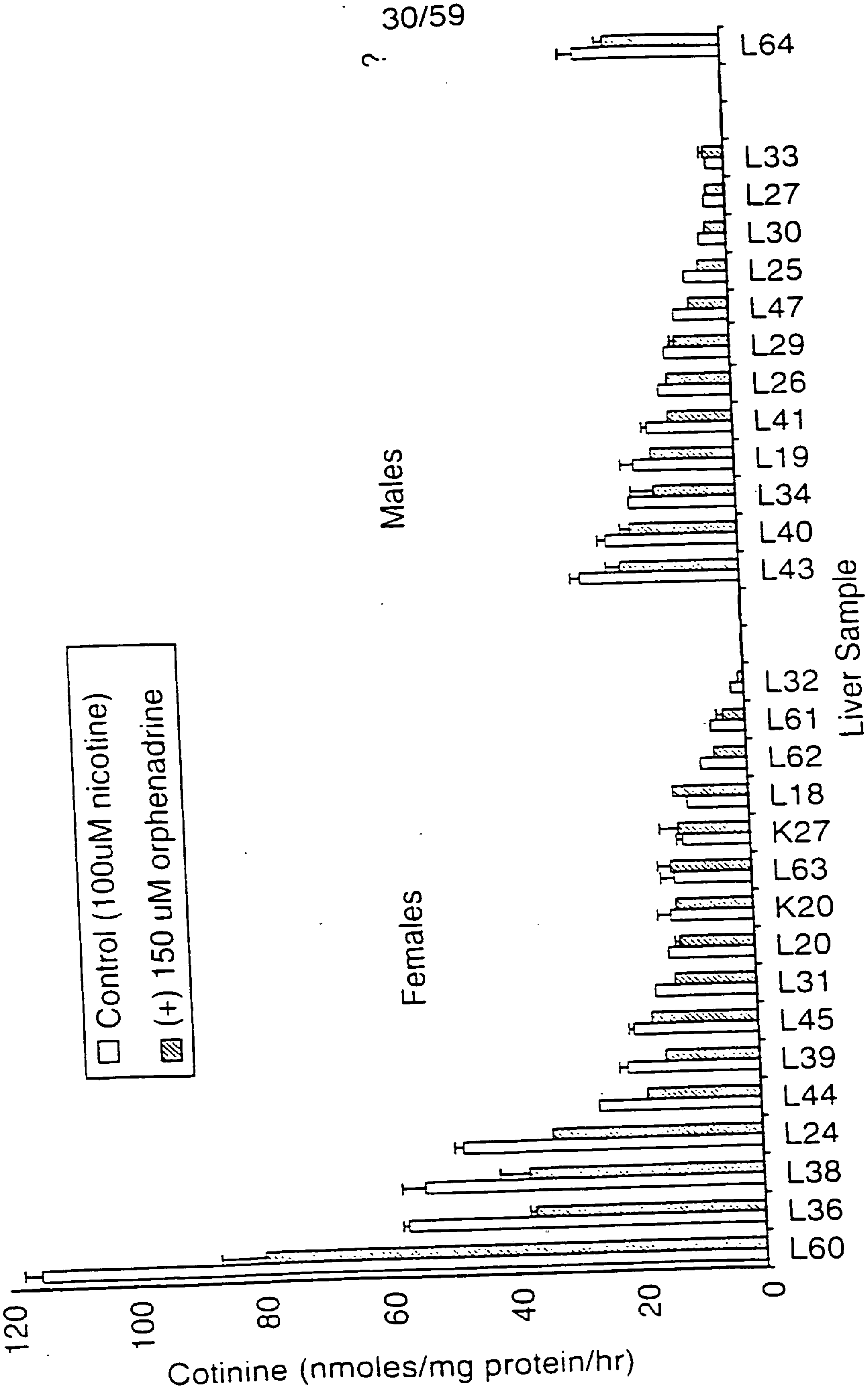

Control (100uM nicotine)
(+) 150 uM orphenadrine
Females
Males
L64
L33
L27
L30
L25
L47
L29
L26
L41
L19
L34
L40
L43
L32
L61
L62
L18
K27
L63
K20
L20
L31
L45
L39
L44
L24
L38
L36
L60
Liver Sample
0
20
40
60
80
100
120
Cotinine (nmoles/mg protein/hr)

FIG.18

09/214851

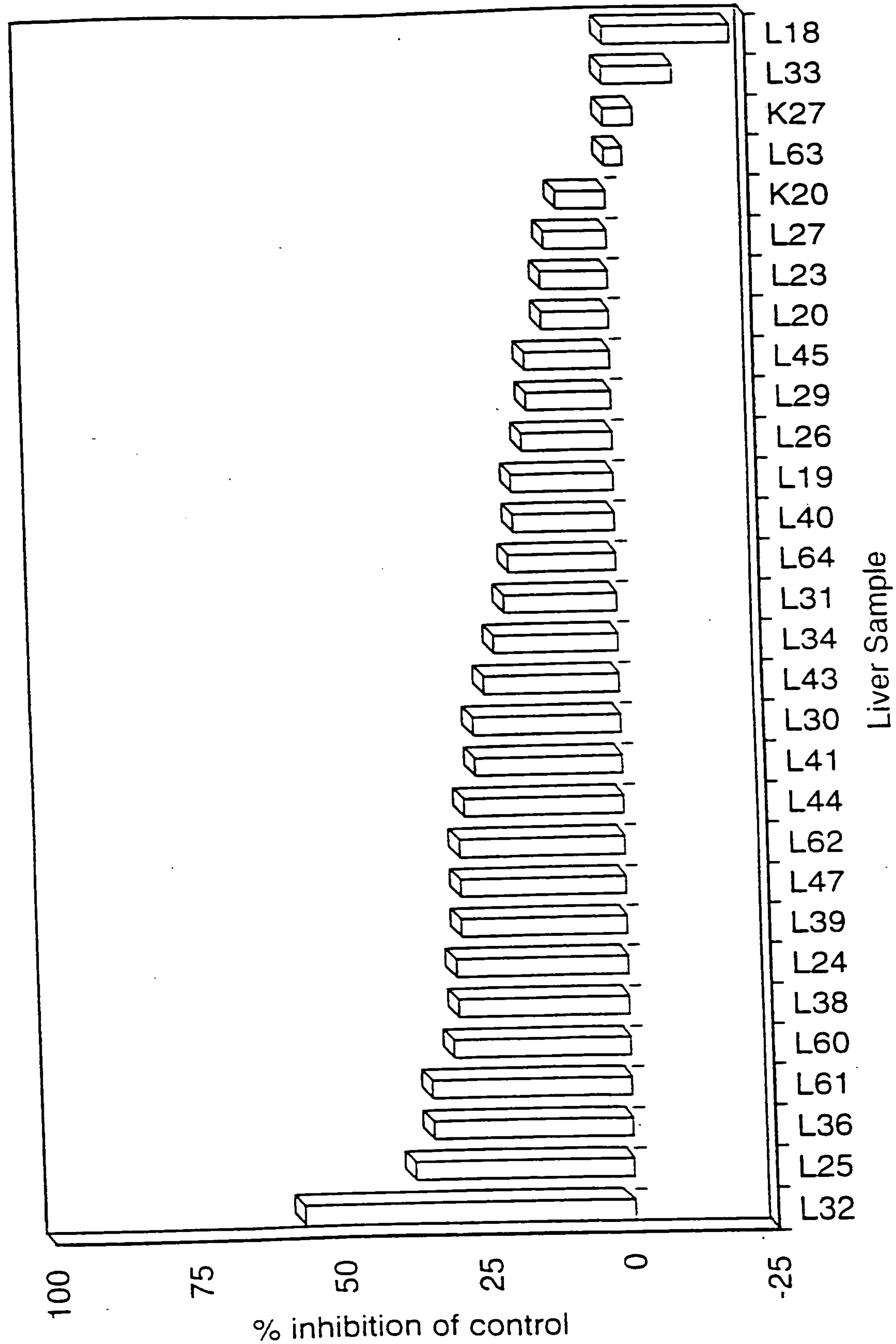


FIG.19

09/214851

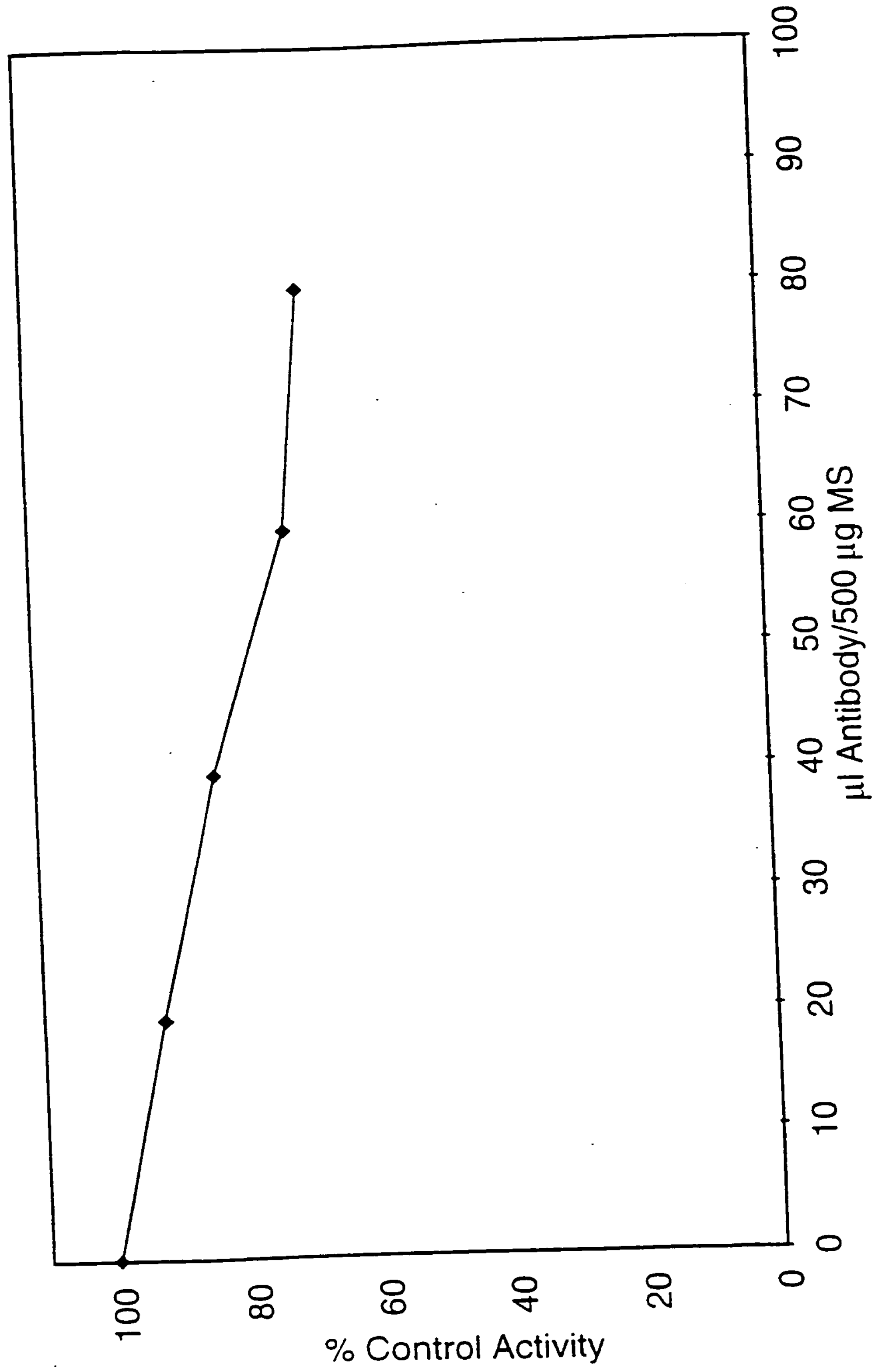


FIG.20

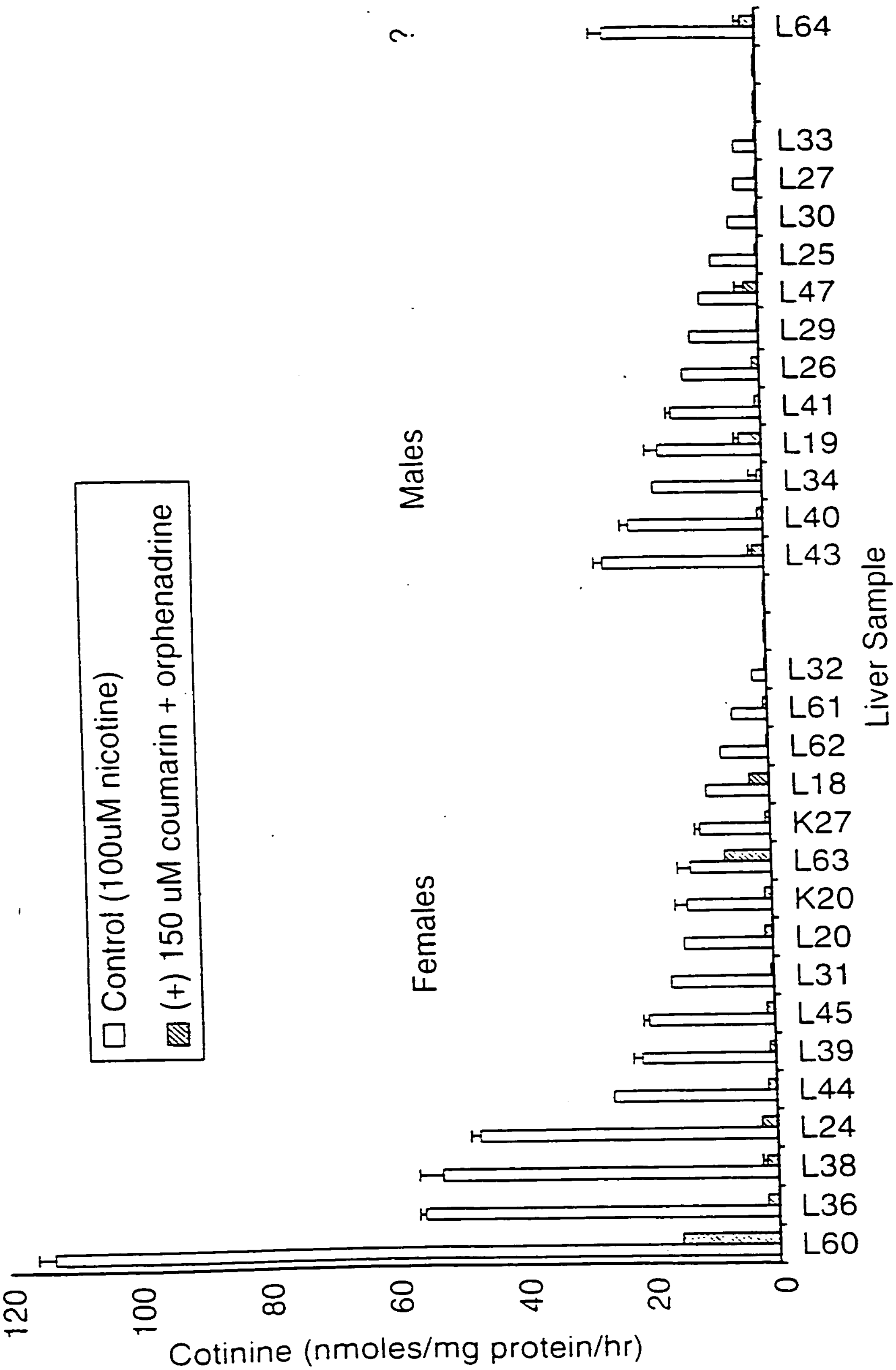

Control (100uM nicotine)
(+) 150 uM coumarin + orphenadrine
Males
Females
L64
L33
L27
L30
L25
L47
L29
L26
L41
L19
L34
L40
L43
L32
L61
L62
L18
K27
L63
K20
L20
L31
L45
L39
L44
L24
L38
L36
L60
Liver Sample
120
100
80
60
40
20
0
Cotinine (nmoles/mg protein/hr)

FIG.21

09/214851

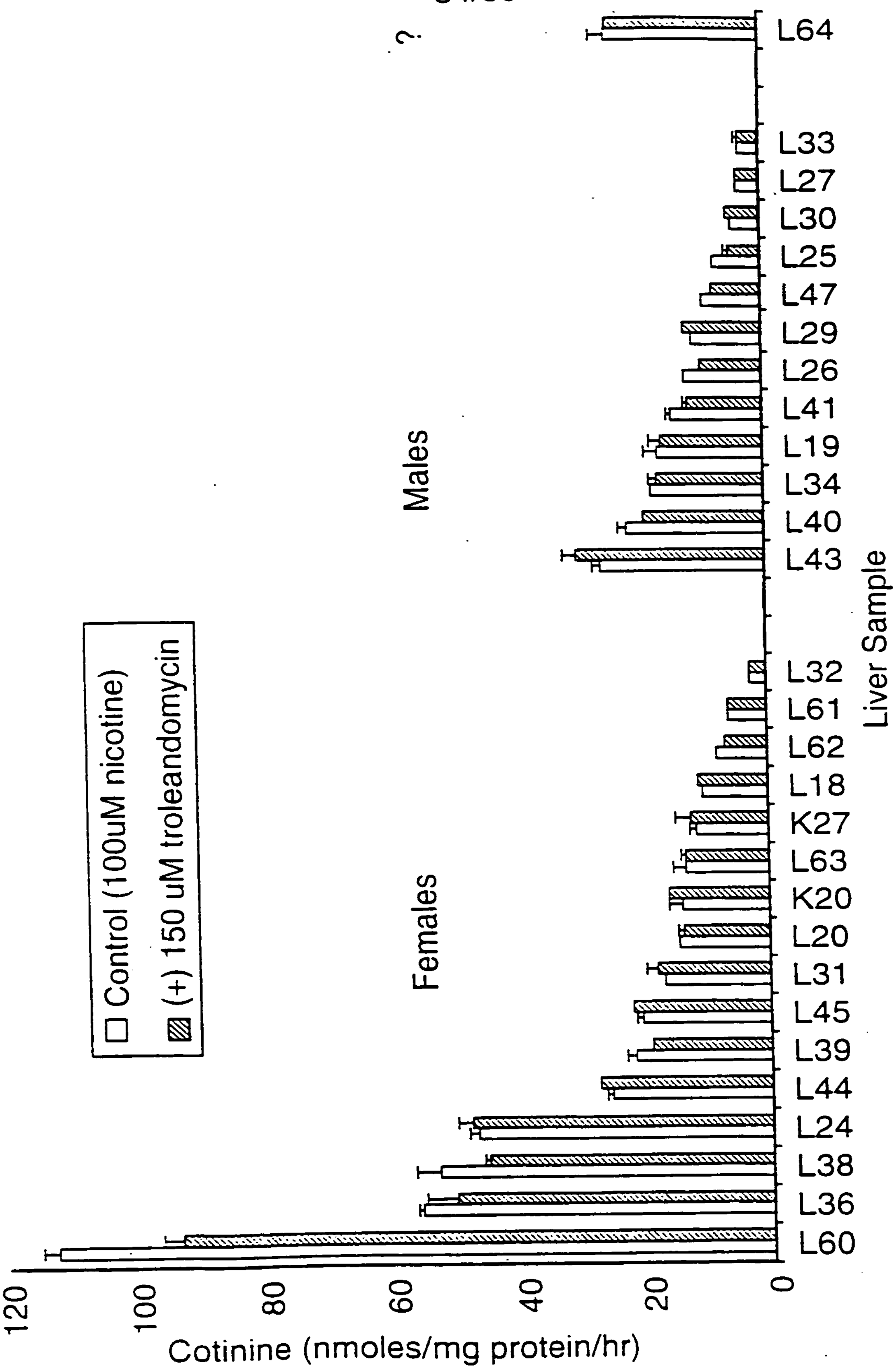
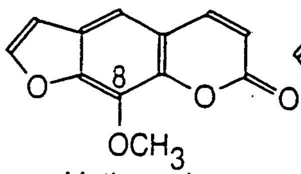
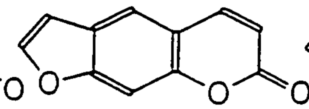


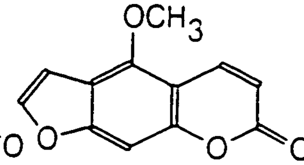
FIG.22



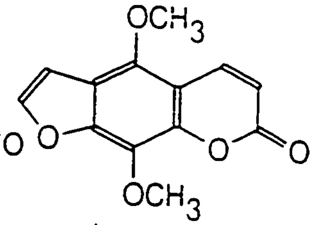
Methoxsalen
ic50 5.4



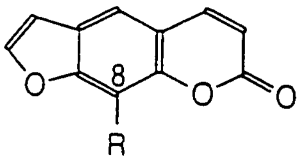
Psoralen
IC50 110



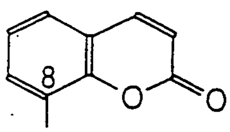
Bergapten
IC50>500



Isopimpinellin
IC50>500

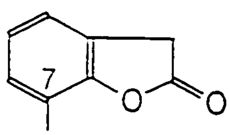


R= $-OCH_2CH_3$	$-CH_2CH$	$-OH$	$-C_6H_5$
$-OCH_2CH_2CH_3$	$-CH_2CH_2CH_3$	$-NH_2$	
$-OCH_2CH_2CH_4CH_3$	$-CH_2CH_2CH_2CH_3$	$-NO_2$	



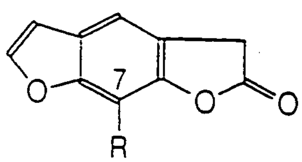
R (coumarin R-H)

R= $-OCH_3$	$-CH_3$		
$-OCH_2CH_3$	$-CH_2CH_3$	$-OH$	$-C_6H_5$
$-OCH_2CH_2CH_3$	$-CH_2CH_2CH_3$	$-NH_2$	
$-OCH_2CH_2CH_2CH_3$	$-CH_2CH_2CH_2CH_3$	$-NO_2$	



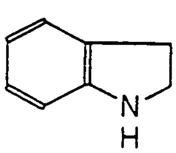
R (2-coumaranone R=H)

R= -H			
$-OCH_3$	$-CH_3$	$-OH$	$-C_6H_5$
$-OCH_2CH_3$	$-CH_2CH_3$	$-NH_2$	
$-OCH_2CH_2CH_3$	$-CH_2CH_2CH_3$	$-NO_2$	
$-OCH_2CH_2CH_2CH_3$	$-CH_2CH_2CH_2CH_3$		

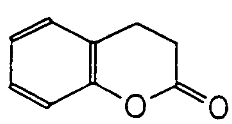


R= $-OCH_3$ (7-methocy -4',5',-5',6-coumaranone)

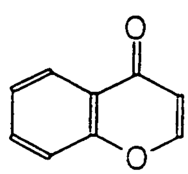
	$-CH_3$	$-OH$	$-C_6H_5$
$-OCH_2CH_3$	$-CH_2CH_3$	$-NH_2$	
$-OCH_2CH_2CH_3$	$-CH_2CH_2CH_3$	$-NO_2$	
$-OCH_2CH_2CH_2CH_3$	$-CH_2CH_2CH_2CH_3$		



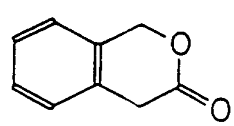
Indole



Dihydrocoumarin

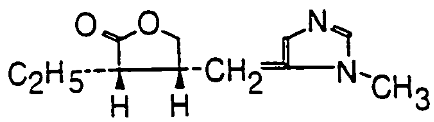


Chomone

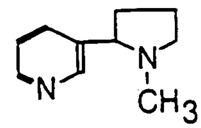


3-Isochromanone

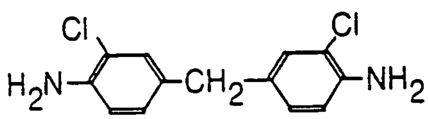
FIG.23A



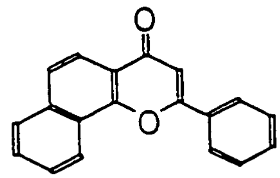
Pilocarpine



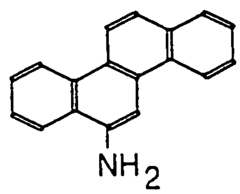
Nicotine



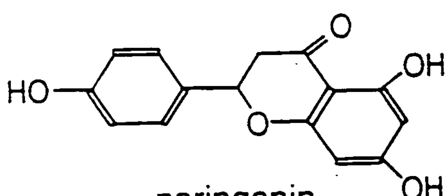
4,4'-Methylene bis[2-chloroaniline



6-Aminochrysene



α-Naphthoflavone



naringenin

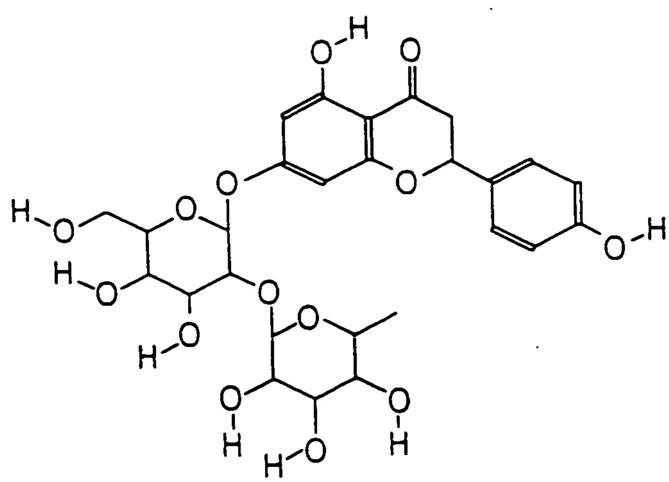
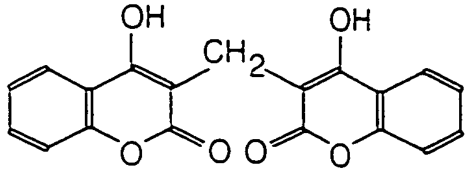


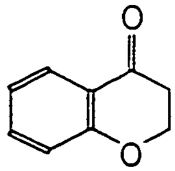
FIG.23B

09/214851

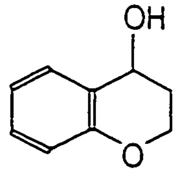


Dicumarol

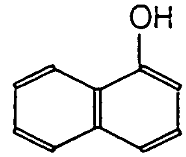
About 80% activity left at 0.05 mM concentration



4-Chromanone

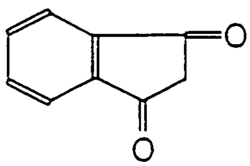


4-Chromanol

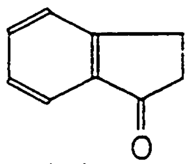


1-Naphthol

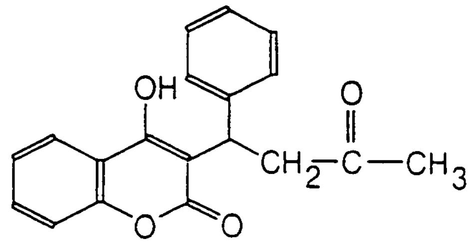
70% inhibition at
0.5 mM concentration



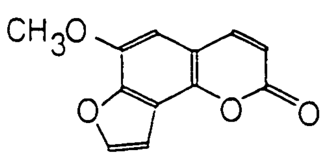
1,3-Indandione



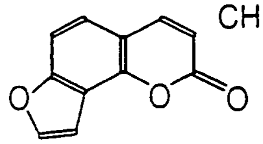
1,Indanone



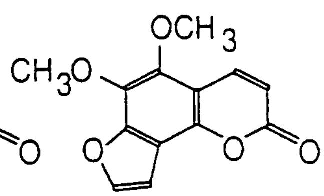
Warfarin



Sphondin
IC50 90



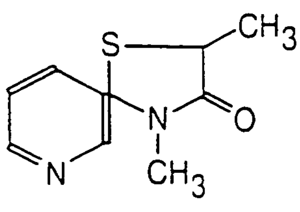
Amgelicin
IC 50 160



Pimpinrllin
IC50>500

$(CH_3CH_2)_2NCS_2NH_4$

Diethyldithiocarbamic acid ammonium salt



SM-12502

$[(CH_3)_2N]_3P(O)$

Hexamethylphosphoramide

$(CH_3)_2NNO$

N-Nitrosodimethylamine

FIG.23C

FIG.24A

The SAS System
Experiment BC1; Pharmacokinetics of nicotine
Revised analysis of kinetics based on re-assays
Does treatment affect AUC?

-------------------------- Compound assayed=COTININE --------------------------

Dependent Variable: AUC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	10578731978	151124745	4.66	0.0397
Error	6	1944298022	324049670		
Corrected Total	13	12523030000			

R-Square	C.V.	Root MSE	AUC Mean
0.844742	19.80871	18001.38	90876.07

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SUBJ	6	10548143898	1758023983	5.43	0.0294
TREATMNT	1	30588081	30588081	0.09	0.7690

Least Squares Means

TREATMNT	AUC LSMEAN
Methoxsalen10-50	92354.2010
Placebo	89397.9447

FIG.24B

The SAS System
Experiment BC1; Pharmacokinetics of nicotine
Revised analysis of kinetics based on re-assays
Does treatment affect AUC?

------------------------- Compound assayed=NICOTINE -------------------------

Dependent Variable: AUC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	54879492.87	7839927.55	5.14	0.0317
Error	6	9143654.02	1523942.34		
Corrected Total	13	64023146.88			

R-Square	C.V.	Root MSE	AUC Mean
0.857182	17.22829	1234.481	7165.426

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SUBJ	6	23085554.55	3847592.43	2.52	0.1422
TREATMNT	1	31793938.32	31793938.32	20.86	0.0038

Least Squares Means

TREATMNT	AUC LSMEAN
Methoxsalen10-50	8672.40779
Placebo	5658.44323

09/214851

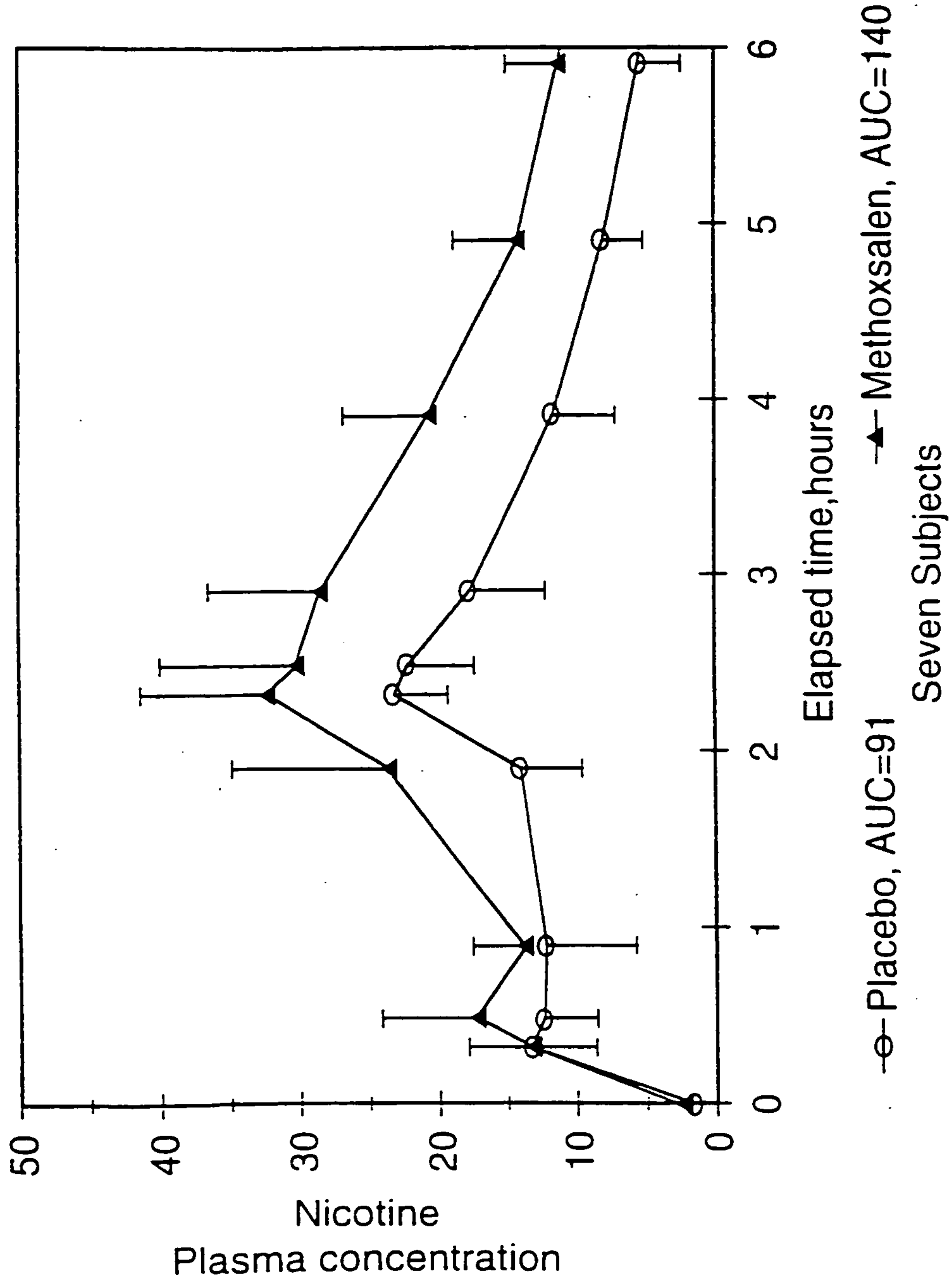


FIG.25

09/214851

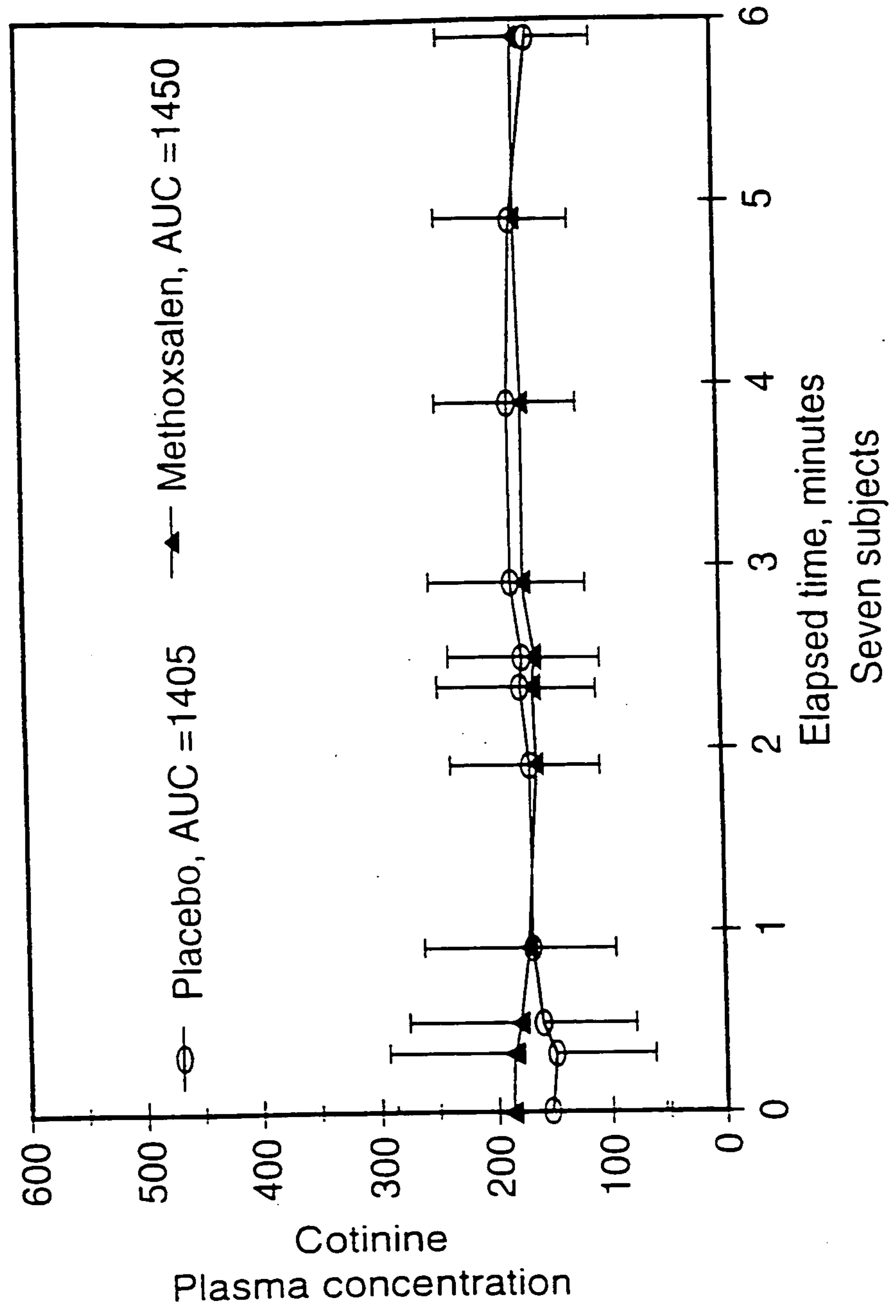


FIG.26

09/214851

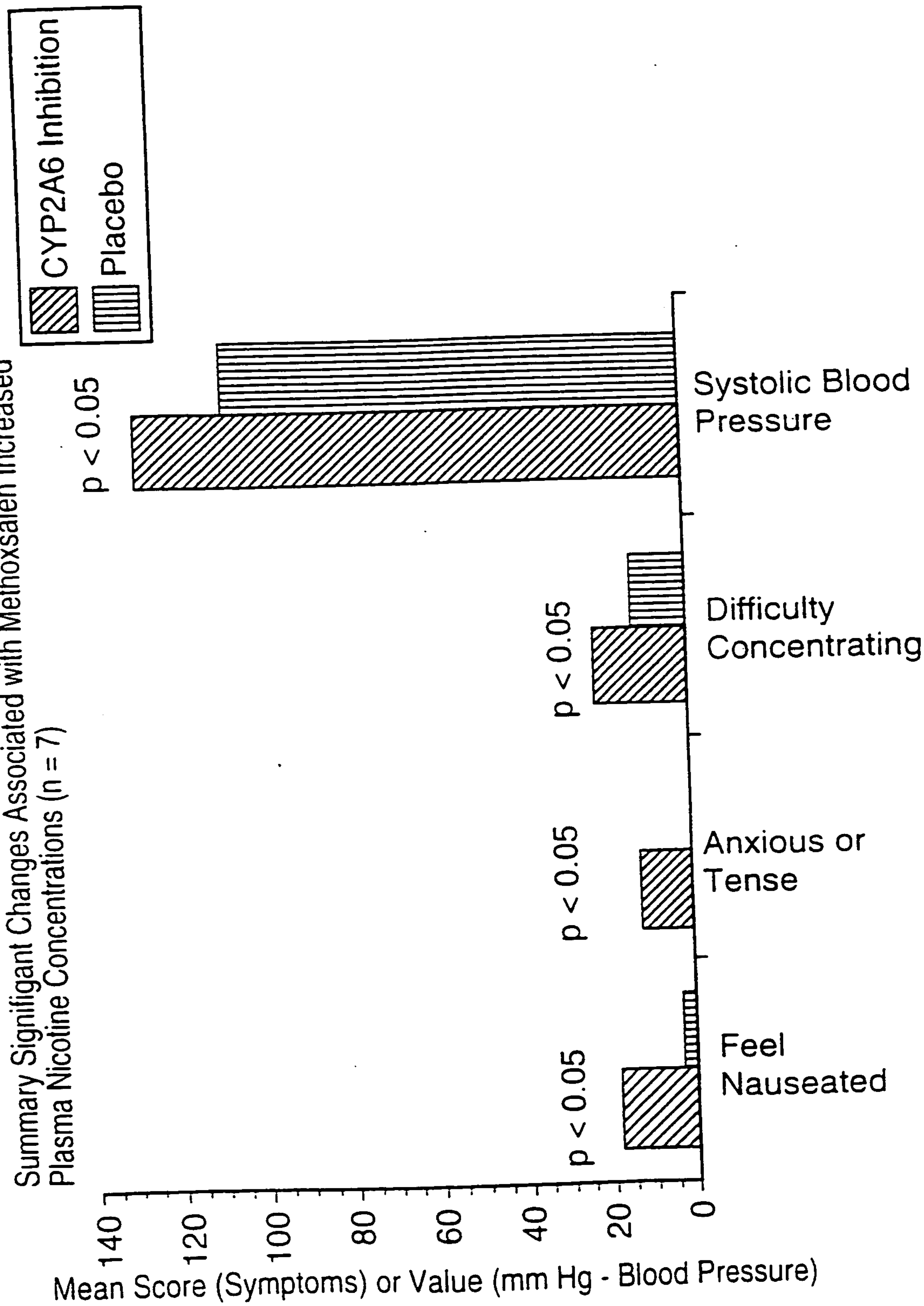


FIG.27

09/214851

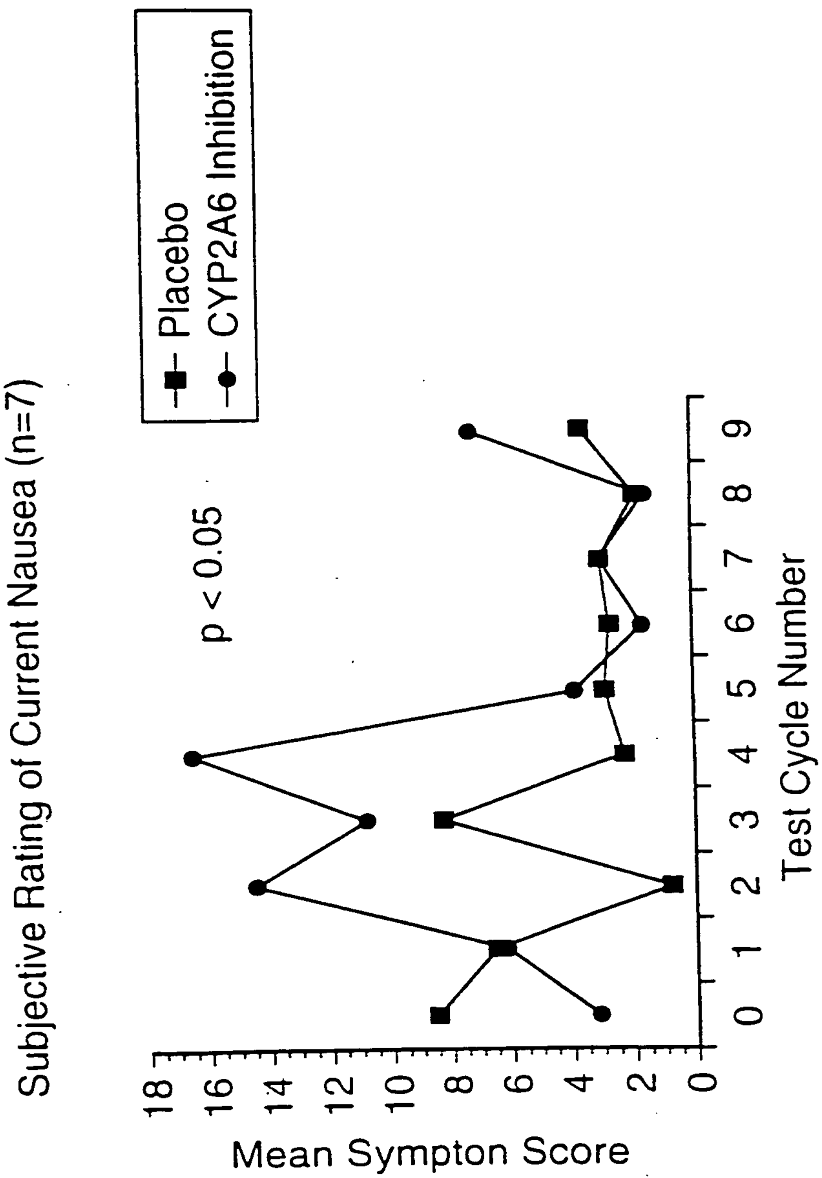


FIG.28A

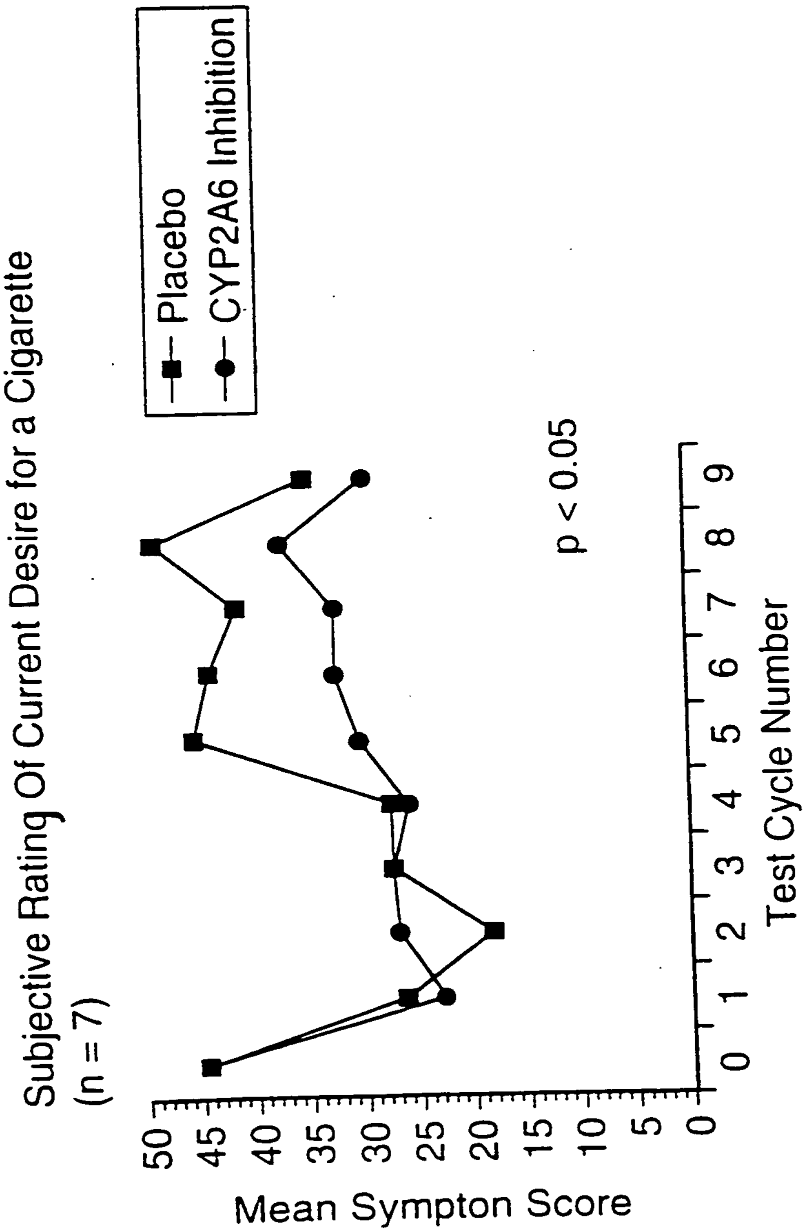

Subjective Rating Of Current Desire for a Cigarette
(n = 7)
Placebo
CYP2A6 Inhibition
p < 0.05
Mean Sympton Score
50 45 40 35 30 25 20 15 10 5 0
Test Cycle Number
0 1 2 3 4 5 6 7 8 9

FIG.28B

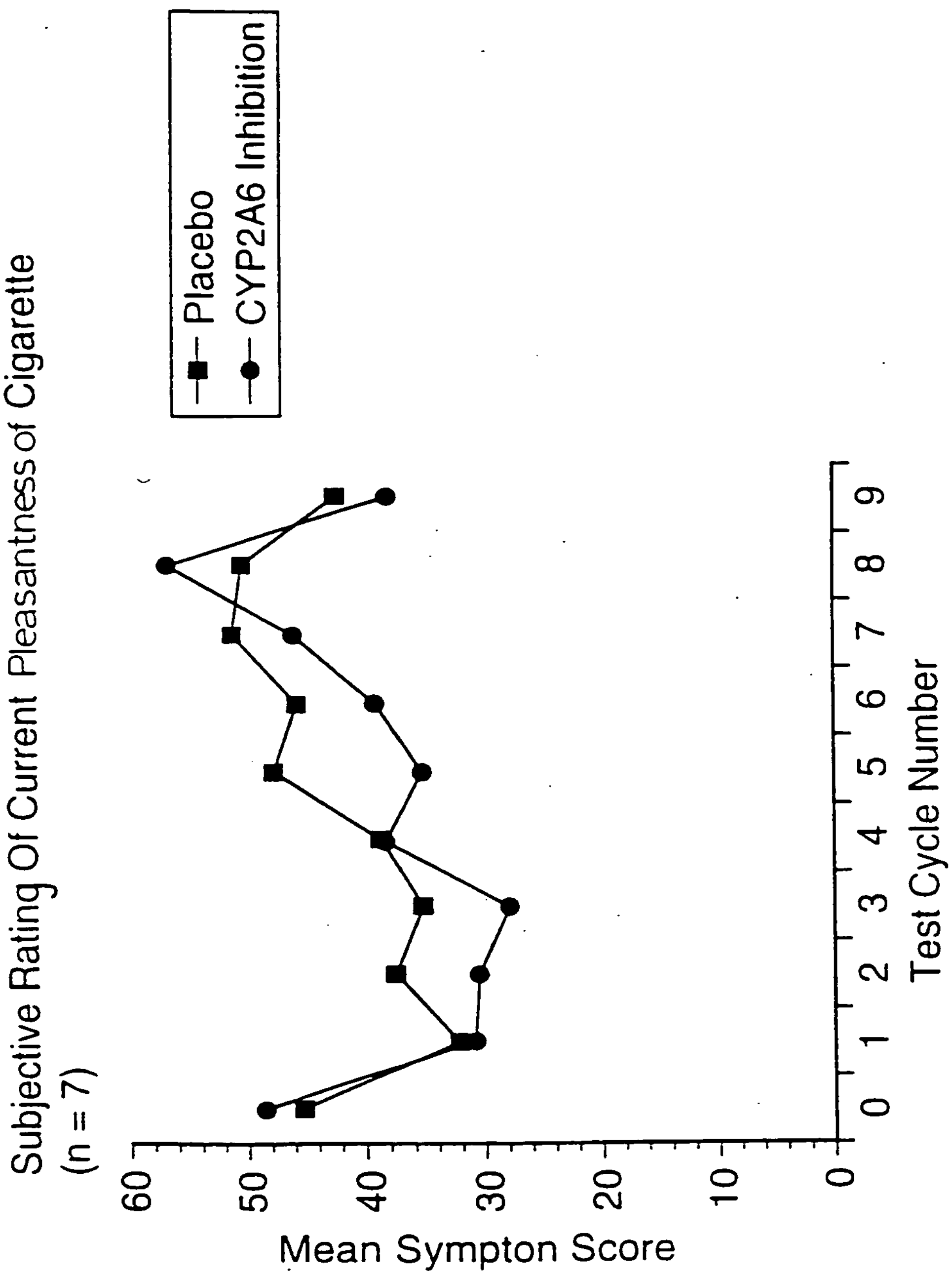

Subjective Rating Of Current Pleasantness of Cigarette
(n = 7)
Placebo
CYP2A6 Inhibition
Mean Sympton Score
Test Cycle Number
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9

FIG.28C

09/214851

09905083.121599

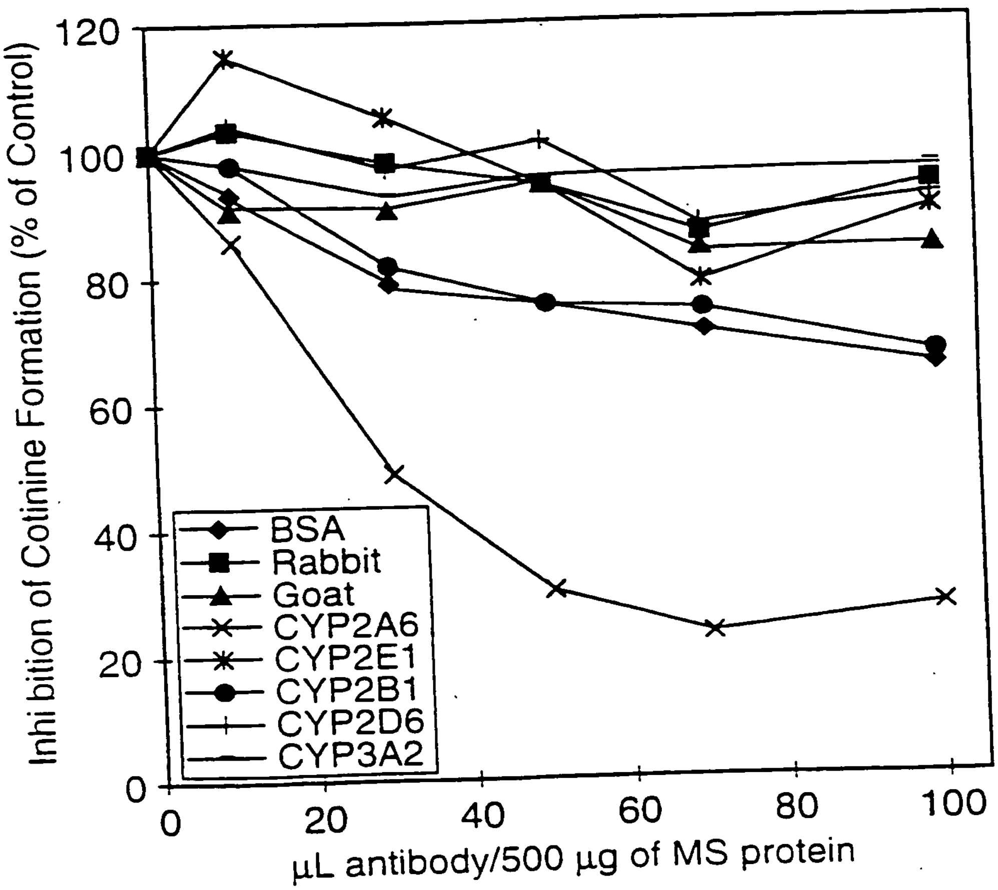


FIG.29

Inhibition of Nicotine to Cotinine Metabolism by various Compounds

Inhibitor	Ki	% Inhibition at 10 uM	% Inhibition at 100 uM	% Inhibition at 150 uM
coumarin	2 uM (n+4)	65 (n=1)	90 (n=1)	85 +/- 11 (SD, n=31)
7-methoxycoumarin	2.5 uM (n=1)	40 (n=1)	60 (n=3)	———
7-methylcoumarin	15 uM*	20 (n=1)	70 (n=3)	———
7-ethoxycoumarin	>100 uM*	10 (n=1)	20 (n=3)	———
7-hydroxycoumarin	200 uM	———	25 (n+1	———
diethyldithiocarbamic acid	14.5 uM (n=1)	———	———	———
pilocarpine	0.1 uM	———	———	———
naringenin	4.3 uM (n=1)	30 (n=1)	70 (n=3)	———
methoxsalen	0.02 uM (n=1)	———	———	———
naringin	.100 uM*	———	10 (n=1)	———
bupropion	———	20 (n=1)	30 (n=1)	———
orphenadrine	———	———	———	20 +/- 16 (SD, n=30)
troleandomycin	———	———	———	3 +/- 11 (SD, n=30)

all nicotine concentrations were at the Km value for cotinine formation in their respective livers
** estimated from screening studies with 10 and 100 uM inhibitor concentrations*

FIG.30A

Ki Values for the Inhibition of the CYP2A6 Substrate Coumarin to 7-Hydroxycoumarin Metabolism by various compound

Inhibitor	Human liver	Monkey liver
methoxsalen	0.29 uM	1.69 uM
nicotine	100.1 uM	24.1 uM
pilocarpine	0.9 uM	0.9 uM

FIG.30B

Effect of Various Compounds on Cotinine Formation

Inhibitor	% control cotinine formation 10 uM	100 uM
coumarin	35	10
naringenin	70	30
7-methylcoumarin	80	30
7-methoxycoumarin	60	40
bupropion	80	70
7-ethoxycoumarin	90	80

FIG.30C

09/214851

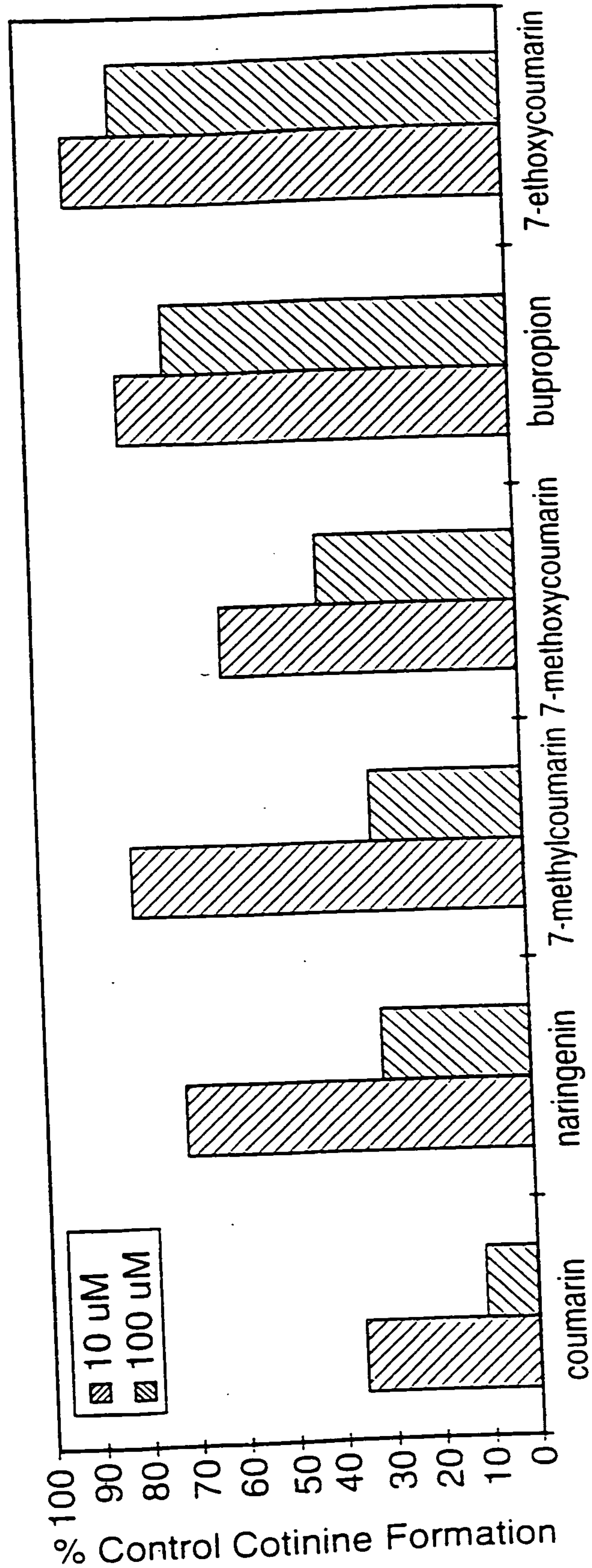


FIG.30D

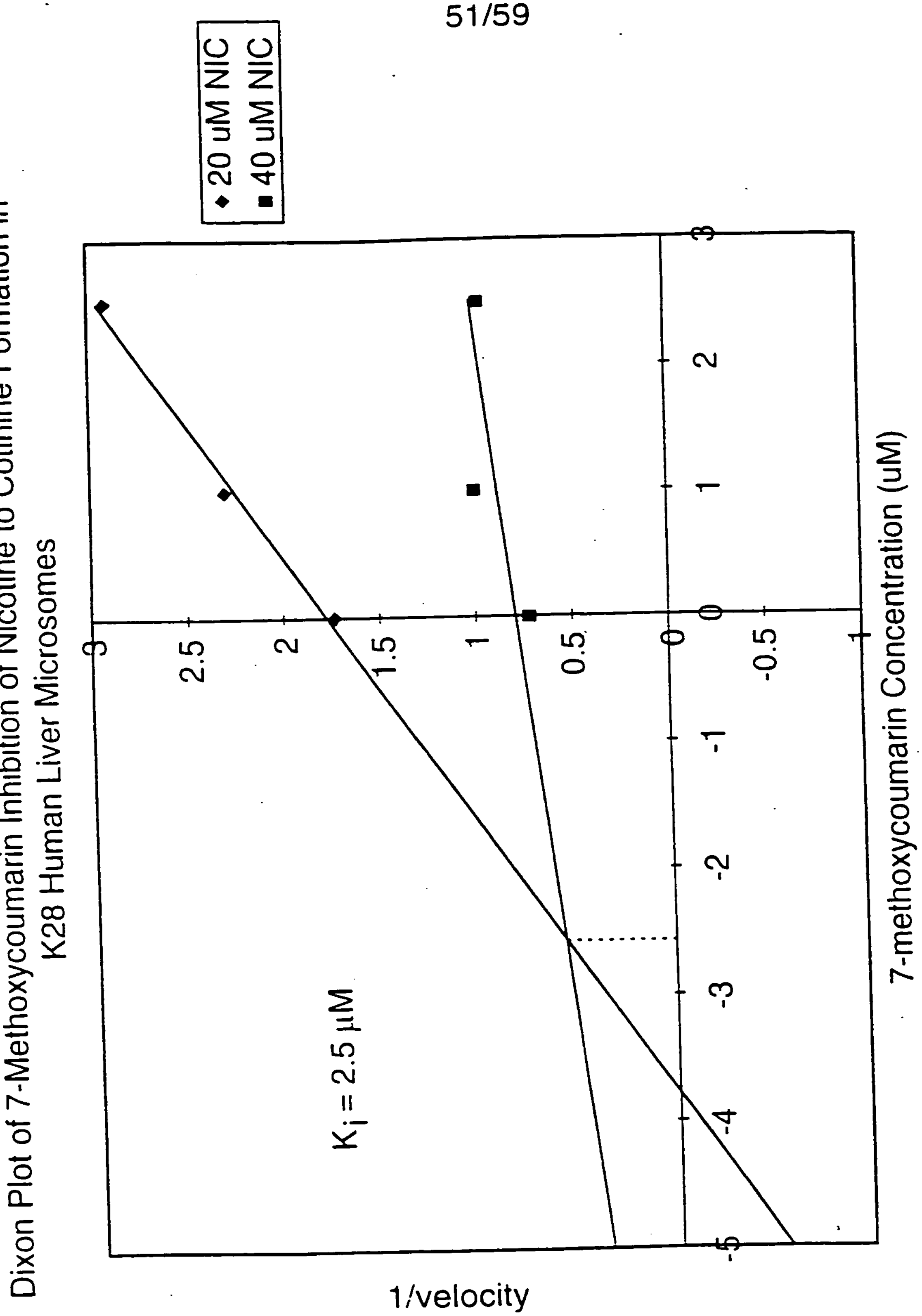

Dixon Plot of 7-Methoxycoumarin Inhibition of Nicotine to Cotinine Formation in K28 Human Liver Microsomes
◆ 20 uM NIC
■ 40 uM NIC
$K_i = 2.5\ \mu M$
1/velocity
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-5
-4
-3
-2
-1
0
1
2
3
7-methoxycoumarin Concentration (uM)

FIG.31

09/214851

Dixon Plot of Methoxsalen Inhibition of Nicotine to Cotinine Formation with 10 Minute Preincubation in K28 Human Liver Microsomes

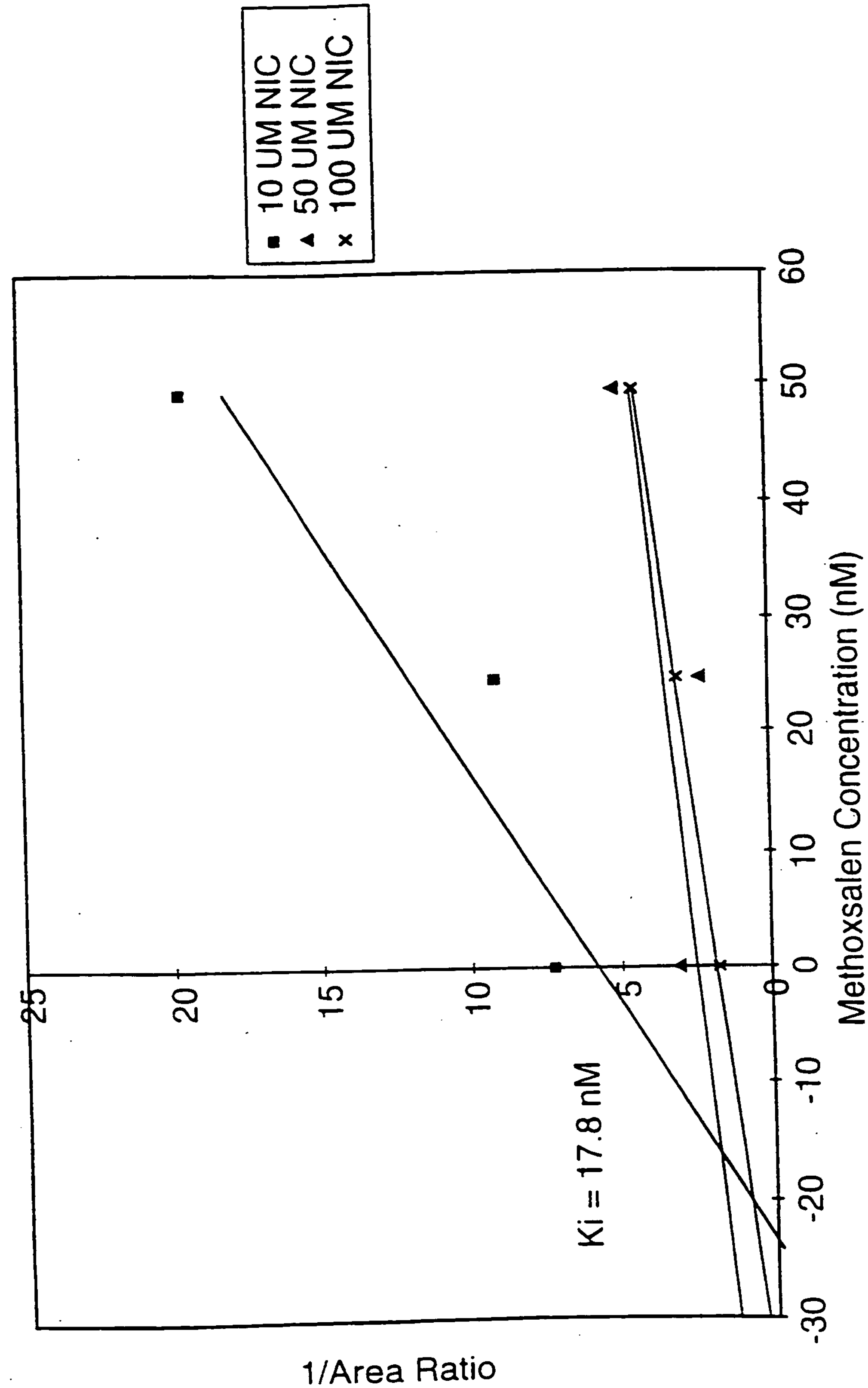


FIG.32

Cornish-Bowden Plot of Methoxsalen Inhibition of Nicotine to Continine Formation with 10 Minute Pre-incubation in K28 Human Liver Microsomes

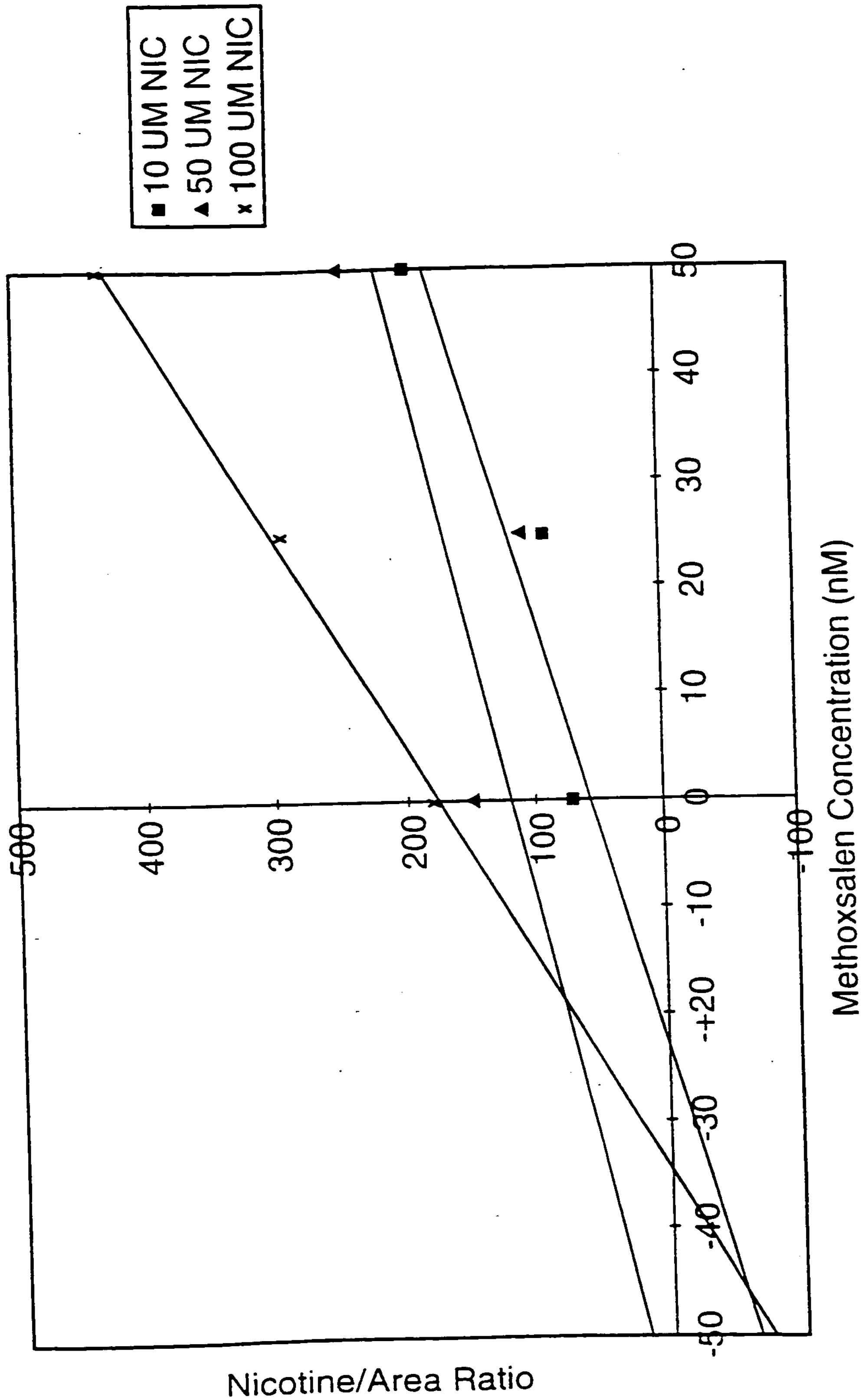


FIG.33

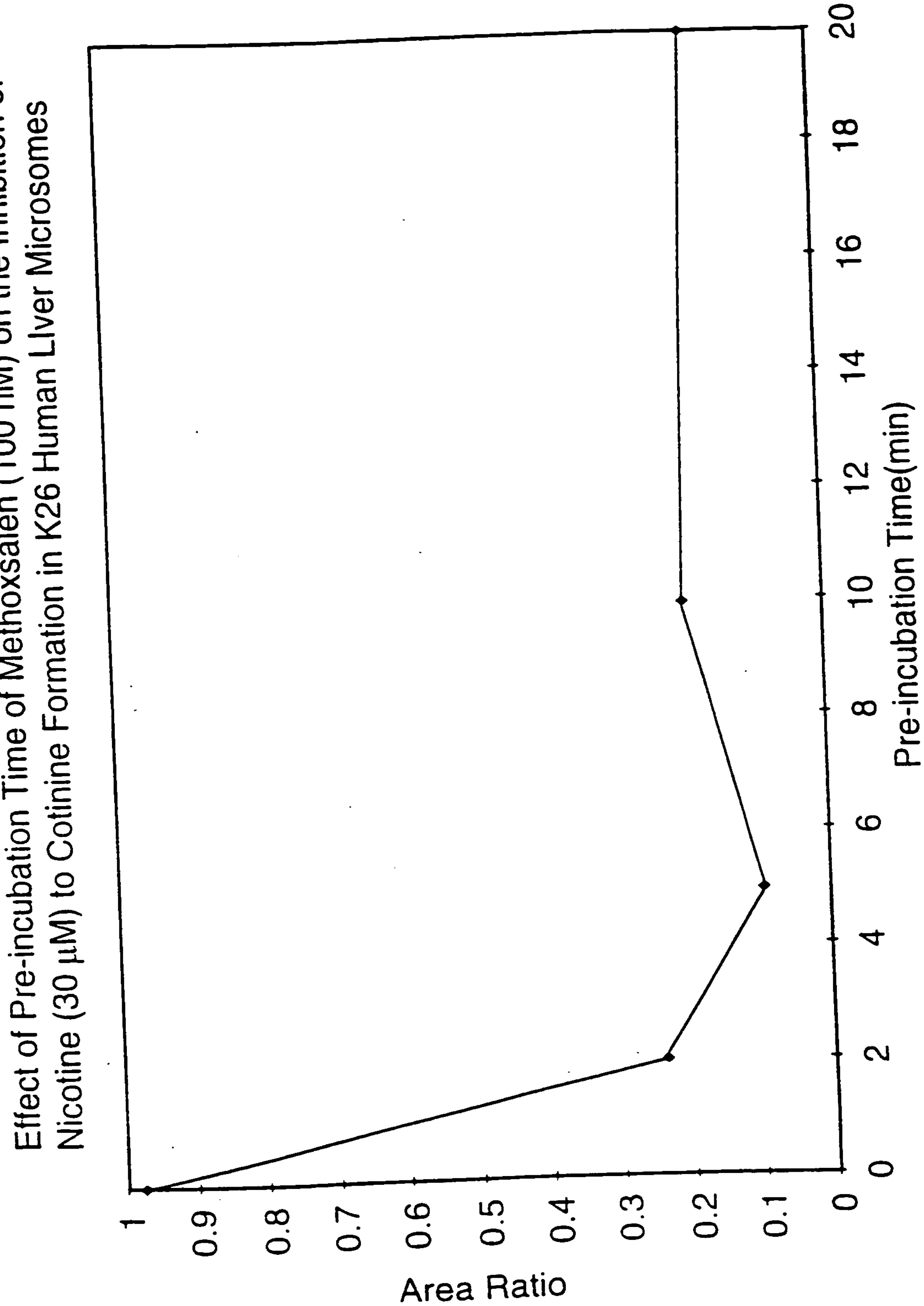

Effect of Pre-incubation Time of Methoxsalen (100 nM) on the Inhibition of Nicotine (30 μM) to Cotinine Formation in K26 Human Liver Microsomes
Area Ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
18
20
Pre-incubation Time(min)

FIG.34

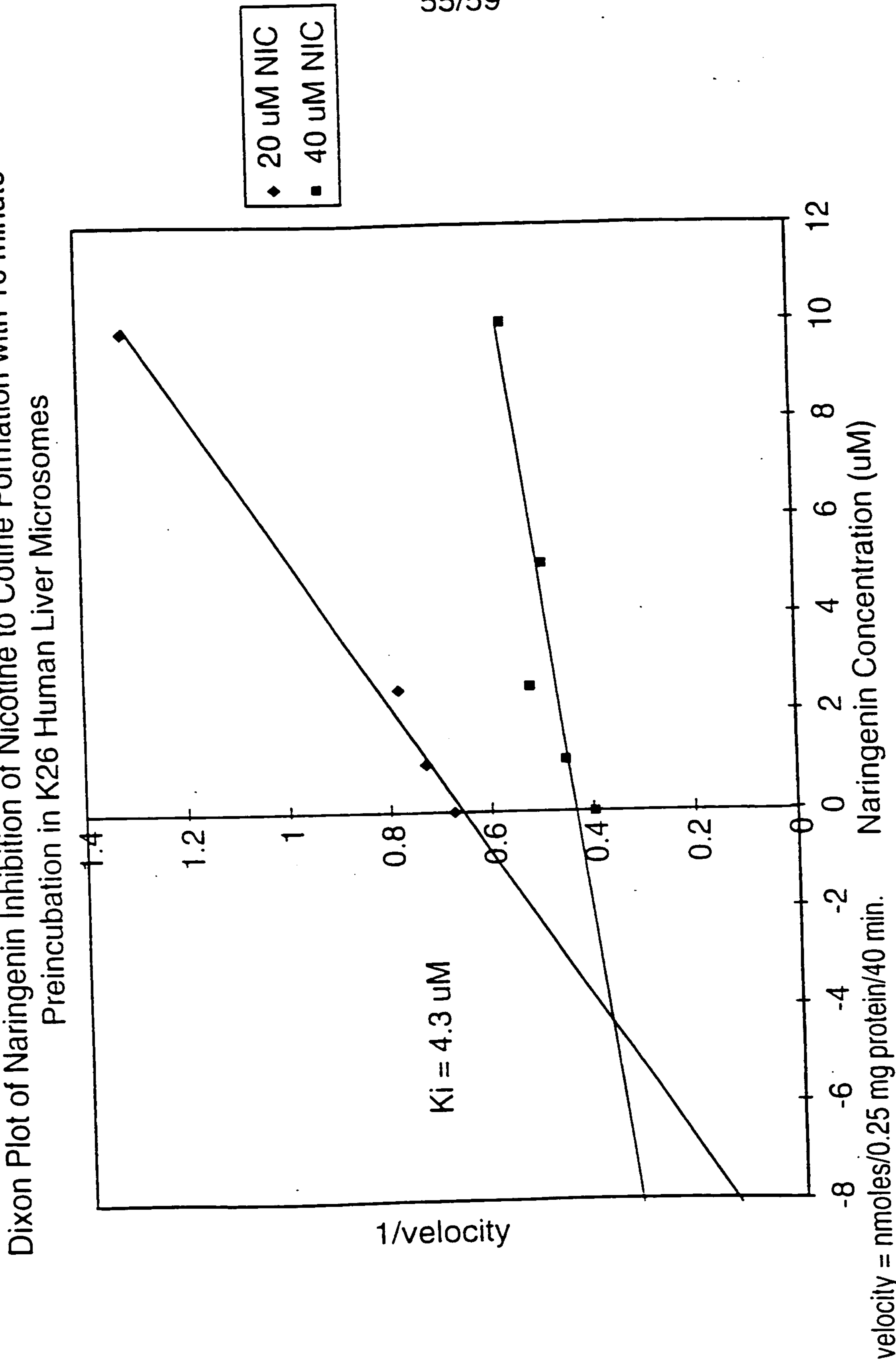

Dixon Plot of Naringenin Inhibition of Nicotine to Cotine Formation with 10 minute Preincubation in K26 Human Liver Microsomes
20 uM NIC
40 uM NIC
1/velocity
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-8
-6
-4
-2
0
2
4
6
8
10
12
Ki = 4.3 uM
Naringenin Concentration (uM)
velocity = nmoles/0.25 mg protein/40 min.

FIG.35

09/214851

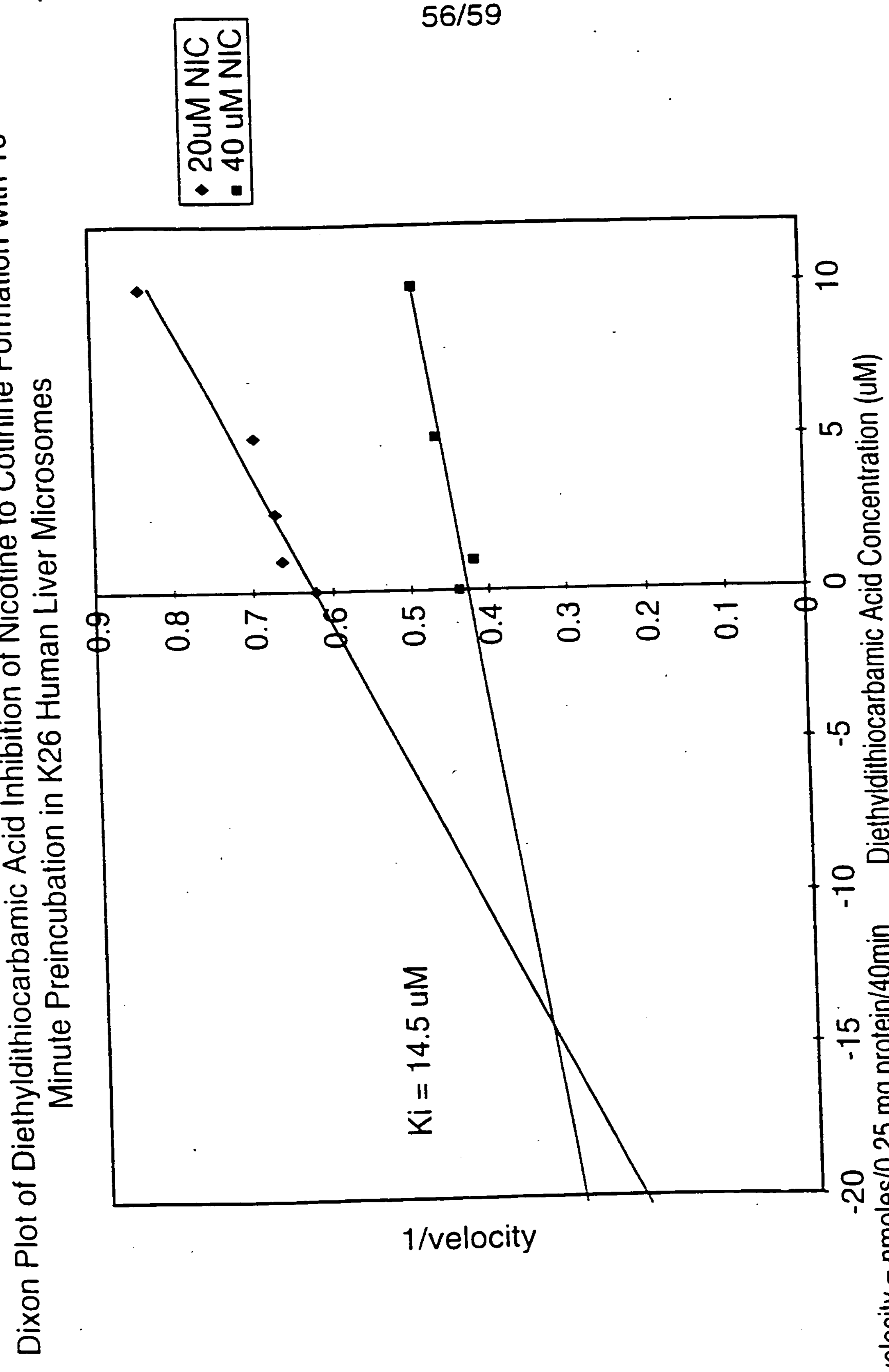


FIG.36

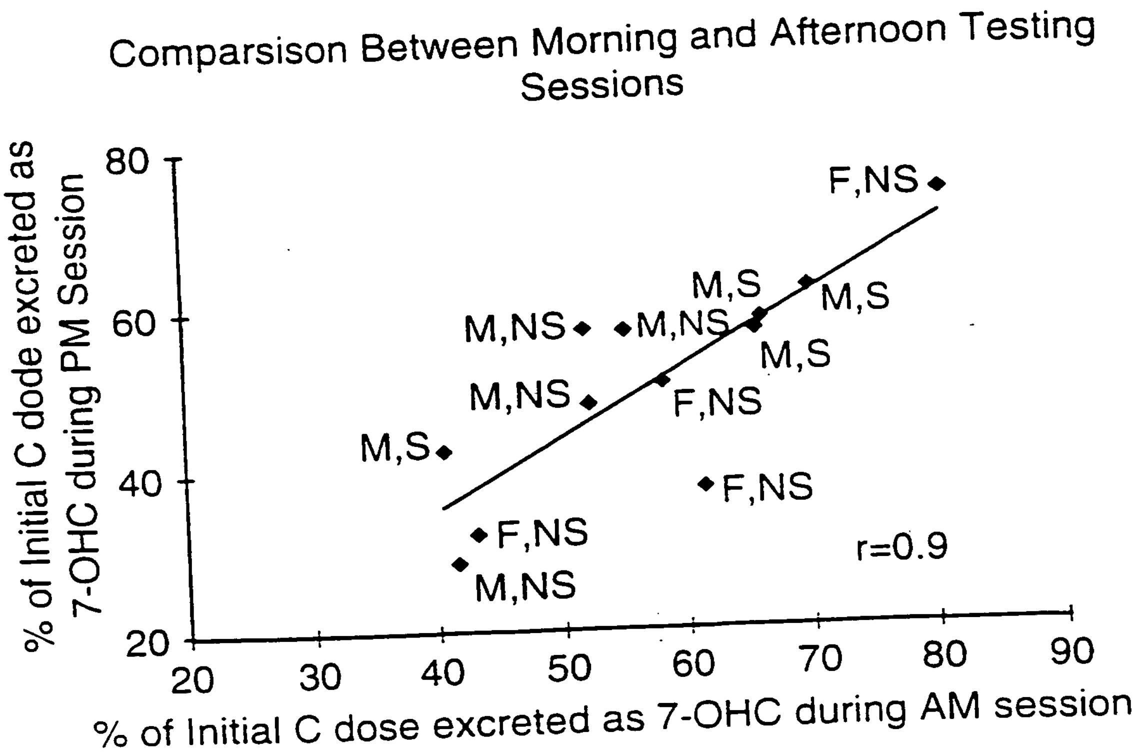

Comparsison Between Morning and Afternoon Testing Sessions
% of Initial C dode excreted as 7-OHC during PM Session
% of Initial C dose excreted as 7-OHC during AM session
80
60
40
20
20
30
40
50
60
70
80
90
F,NS
M,S
M,S
M,NS
M,NS
M,S
M,NS
F,NS
M,S
F,NS
F,NS
M,NS
r=0.9

FIG.37

09/214851

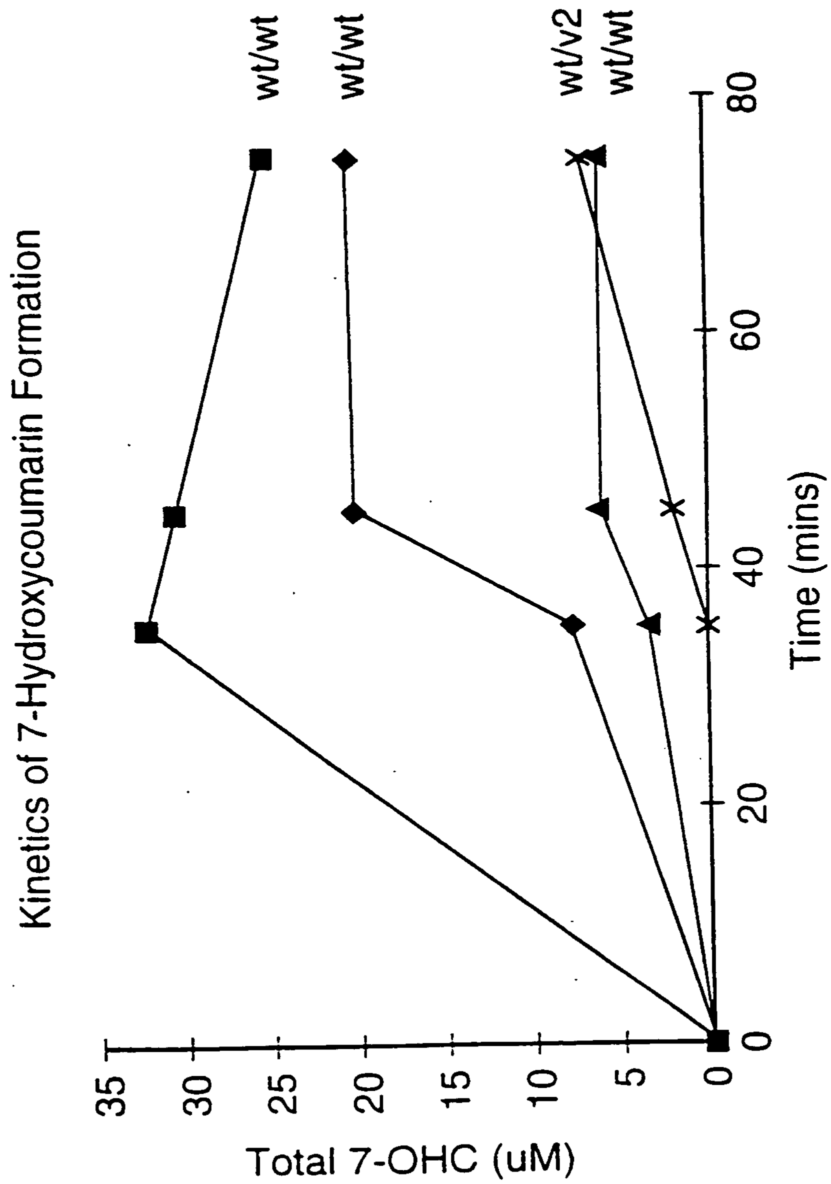


FIG.39

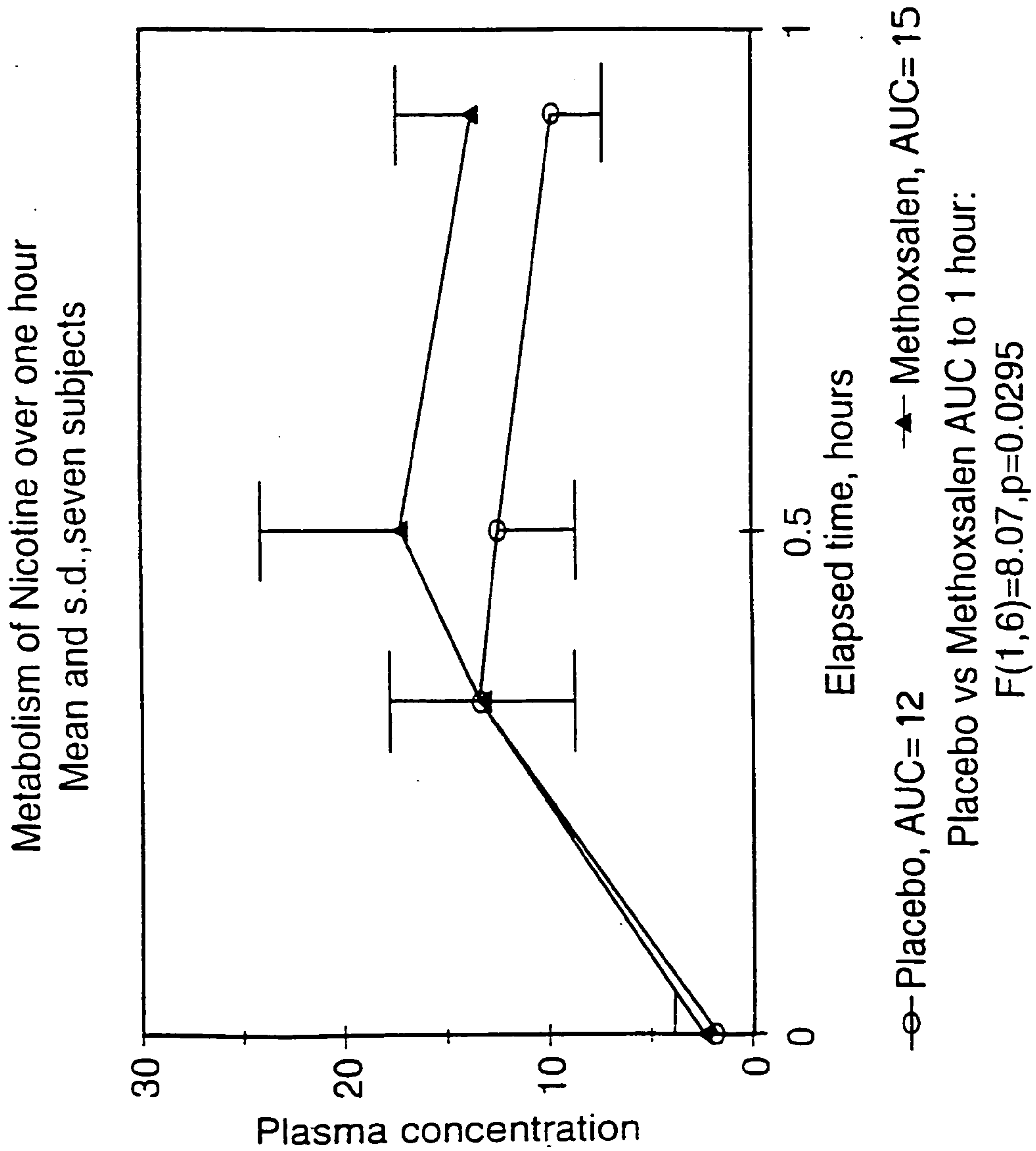

Metabolism of Nicotine over one hour
Mean and s.d.,seven subjects
30
20
10
0
Plasma concentration
0
0.5
1
Elapsed time, hours
Placebo, AUC= 12
Methoxsalen, AUC= 15
Placebo vs Methoxsalen AUC to 1 hour:
F(1,6)=8.07,p=0.0295

FIG.38